COMPARATIVE STUDY ON PATHOLOGICAL CHANGES IN THE SMALL INTESTINE OF SHEEP AND GOAT EXPERIMENTALLY INFECTED WITH *TRICHOSTRONGYLUS COLUBRIFORMIS*

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Comparative Study on Pathological Changes in the Small Intestine of Sheep and Goat Experimentally Infected With *T. Colubriformis*

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I here declared that the experimental research work of the MVSc thesis entitled "Comparative Study on Pathological Changes in the Small Intestine of Sheep and Goat Experimentally Infected with *T. Colubriformis*" in partial fulfillment of the requirements for the degree of Master of Science in veterinary pathology is my original work and has not been submitted for any of the other degree or diploma of any university, nor the data have been derived from any thesis or article of any university or scientific journals. The source of resource used and all assistance obtained during the course of investigation have been duly acknowledged.

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DEDICATION

I dedicated this thesis manuscript to my beloved father, Tafere Bogale who passed in February 7 2010 G.C for nursing me with affection, love and for his dedicated partnership in my life success, thus I am not lucky to celebrate my under and post graduate achievement with him and to my beloved mother who is special in my life career.

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LIST OF ABBREVIATIONS

BEC	Blood Eosinophil Count
CCK	Cholecystokinin
FL	Femtoliters
GI	Gastrointestinal
IEC	Intestinal Epithelial Cell
IG	Immunoglobulin
IL	Interleukine
Spp	Species
Th	T –Helper
TNF	Tissue Necrosis Factor
T. Colubriformis	*Trichostrongylus Colubriformis*
VFI	Voluntary Feed Intake

ABSTRACT

The experimental study design was conducted to determine hematological and serum biochemical parameters and pathological changes in the small intestine of sheep and goat experimentally infected with *T. colubriformis* in fly-proof experimental facility at College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia. During the study period, 13 sheep (6 controls, 7 infected) and 14 goats (7 controls, 7 infected) were included in the experiment. The infective larvae of *T. culibriformis* (L3) as a single dose of 10,000 per-animal was administered orally to infected sheep and goats (G2 and G4). From the infected group, the total recovered mean worm burden was recorded higher in infected goat (5016 larvae of *T. colubriformis*) (P<0.05) than infected sheep (3446 larvae) with establishment rate of 50.16% and 34.46% respectively. The total mean hematological and biochemical values recorded in infected and uninfected sheep and in infected and uninfected goat were significantly (P<0.05) lower in infected than uninfected control, except RBC and WBC count. In sheep PCV, Hb and albumin were significantly (P<0.05) lower in infected group than uninfected control group. Gross lesions found in the intestine were enteritis with petechial hemorrhages, edema, hyperemia and mucosal slough which were marked in the duodenum (62.69%) and jejunum (33.33%) in sheep and 47.05% duodenum and 45.09% jejunum in goat. The microscopic lesions developed by *T. colubriformis* were subtotal villus atrophy, hemorrhage, straighten and elongated dilated crypts, loss of epithelium, mucosa erosion and infiltration of inflammatory cells. In conclusion, the present experimental investigation showed that *T. colubriformis* infection affected hematological parameters, biochemical indices and caused pathological changes of small intestine in sheep and goat, with more severe infection in goats than sheep although they were under the same management condition.

Key terms: *Control, experimental infection*, *intestine, lesion, sheep and goat, T. colubriformis*

1. INTRODUCTION

Parasitic diseases are worldwide concern and considered as one of an obstacle in the health and product performance of animals (Horal, 2006). Animals are impressed by multifarious gastrointestinal parasites such as nematodes, trematodes and cestodes (Sykes, 2004). Gastrointestinal parasitic infections are global problem for small and large scale farmers, but their effect is greater in sub-Saharan Africa in general and Ethiopia in particular due to a wide range of agro ecological factors are available and suitable to diversified hosts and parasitic species (Fikru *et al*., 2006). Domestic ruminants are frequently exposed to multiple parasitic infections throughout their life (Clark, 2001; Cox, 2001). In the field while sharing common pasture, animals are exposed to a variety of parasites among which are gastrointestinal nematodes that cause considerable animal health problems in many parts of the world (Waller *et al*., 2004).

Gastrointestinal nematodes are the most widely distributed helminthes in pasturing small ruminant throughout the world and are regarded as an important health and economic problem of sheep and goat production (Rinaldi *et al*., 2007). They are infections caused by different genera of nematodes (Trichostrongylus, Haemonchus, Teladorsagia, Bunostomum, Oesophagostomum, and Chabertia), each localized in a specific tract of the digestive apparatus of the host. Infection by GI nematodes can be similar to a nutritional disease, since the presence of worms usually induces a decrease in appetite and digestibility of the food and diversion of nutrients from production sites toward the repair of tissue damage caused by parasites (Hoste *et al*., 2006, 2010).

Most infected sheep and goats remain sub clinically infected; others show gastrointestinal pathologies whose intensity depends on the worm burden, the species of nematode, their life cycle (if there are or not include tissue phases) and their localization. Pathological conditions of the animals can be assessed by examination of hematological and

biochemical analysis of blood (Chirkena *et al*., 2016). Haematology has been widely used to provide information about health status in animals. A deviation of certain blood parameters from their normal limits might serve a guide for diagnosis diseases including parasitism (Mal *et al*., 2001). Although general aspects of morphology, biology, epidemiology and control of gastrointestinal nematodes of small ruminants are generally known, others, such as the pathogenic role of these parasites in sheep and goats regarding pathology are less known (Hoste *et al*., 2010). Especially small intestine lesions caused by GI nematodes such as *T. colubriformis* and its effect on hematology and biochemical parameters in sheep and goats have not been studied in detail, and studies on host–parasite interaction in sheep and goats remain few and dispersed (Hoste *et al*., 2010). Thus, the aims of the study were:

- To characterize gross and microscopic lesion in the small intestine of sheep and goats experimentally infected with *T. colubriformis.*
- To compare the effect of *T. colubriformis* on hematological and serum biochemical parameters in experimentally infected sheep and goat.

2. LITERATURE REVIEW

2.1. Trichostrongylosis and Its Cause

Intestinal parasites such as members of the genus Trichostrongylus parasitize the *proximal part of small intestine of ruminants* worldwide. They cause significant inefficiency production in subclinical, or clinical disease known by *diarrhea, ill-thrift, and in some cases, death (*Williams *et al*., 2010). *T. colubriformis, T. vitrinus,* and *T. rugatus* are the most important species infecting sheep and goat and others include *T. longispicularis, T. falculatus, T. capricola,* and *T. probolurus. T. colubriformis* and *T. longispicularis* are also affect cattle. Although some *T. axei* may be found in the duodenum of cattle and sheep, this species is primarily parasitic in the abomasum (Cardia *et al*., 2011). In arid and semiaride zones of eastern Ethiopia, prevalence of *T. colubriformis* in sheep and goat at post-mortem examination are 87.15% and 89.90% respectively. So, *T. colubriformis* is the most common important worm next to *H. contortus* and is the most predominant small intestinal worm. It is very small and threadlike in structure and feeds on nutrients on the surface of the mucosa and interferes with digestive function (Abebe and Esayas, 2001).

2.1.1. Life cycle

The life cycle (figure 1) of Trichostrongylosis is direct, and sexually dimorphic adults are present in the digestive tract, where fertilized females produce large numbers of eggs that are passed in the faeces. *T. colubriformis* eggs usually hatch within 1–2 days. After hatching, the larvae feed on the soil and undergo two moults, then to ensheathed third stage larvae in the environment. The sheath is a cuticular layer used to shed in the transition from stage two (L2) to stage three (L3) and it protects the third stage larvae from environmental conditions but it inhibits the larvae from feeding. Exposure of the

host occurs by ingestion of stage three (Robert *et al*., 2013). During its passage through the abomasal environment, the L3 stage loses its protective sheath and has a tissue phase and establish preferentially in the proximal 5-6 meters of the small intestine of sheep and goat. A small proportion of the parasite infests the abomasal antral mucosa around the pylorus. The larvae enter tunnels above the basal lamina at the base of the villi in the superficial intestinal mucosa and they persist throughout their life time at least partially embedded in the epithelium. Larvae take to develop over a 2-week period into adults with a prepatent period of 16-18 days (Roy *et al.,* 2004).

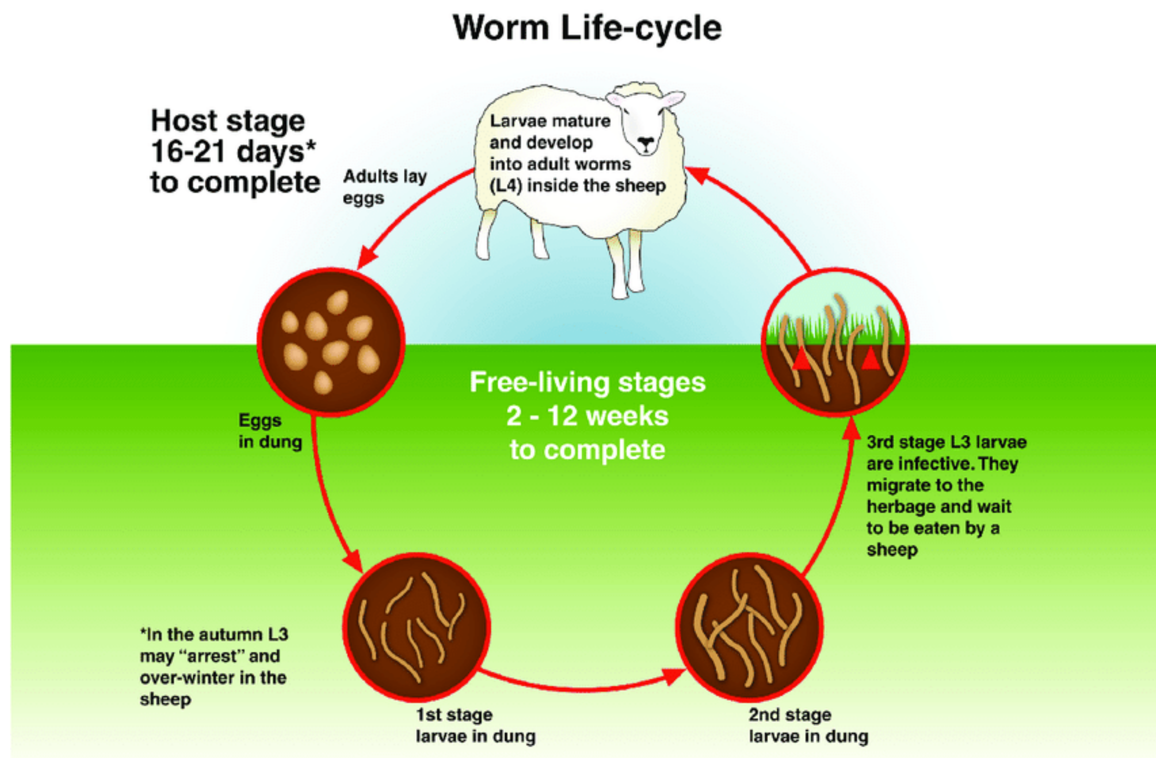

Figure 1: The basic life cycle of the nematode parasite of small ruminants.

Source: Available from: https://www.researchgate.net/figure/ [accessed 26 Jul, 2020].

First, second and third stage larvae in figure 1 are free living in the environment. Disease is caused by the fourth and adult stages of larvae and they are parasitic in the

gastrointestinal tract of the host. It depends on factors such as species of nematode; species, age and health status of the host; intensity of infection; environment and management aspects (Taylor *et al*., 2007).

2.2. Anatomy and Pathology of Intestine

2.2.1. The intestine

The small intestine is a typical tubular organ in that it has all of the typical tunics and layers (figure 2). The tunica mucosa is modified to fulfill the role of absorption. It projects out into the lumen what we called villi and dips down to the underlying lamina muscularis mucosae which are further subdivided into the lamina epithelialis mucosa, the lamina propria and the lamina muscularis mucosa forms pockets called crypts and the folding forms the villi. The villi involve enterocytes, goblet cells in its upper region and neuroendocrine cells which are used for absorption, protective mucus and local hormones production respectively. The crypt of Lieberkuhn contains goblet cells, paneth cells (defensive), neuroendocrine cells, stem cells and intraepithelial lymphocytes. Lamina epithelialis mucosa is made up of simple columnar epithelium with goblet cells. The lamina propria is part of the mucosa and is composed of loose connective tissue rich in blood and lymphatic vessels present in the core of the villi and between crypts. Lamina muscularis mucosa is made up of thin layer of smooth muscle located at the base of the crypts (Dellmann and Brown, 1981).

The second layer is the tunica sub mucosa. This layer blends with the lamina propria and in the duodenum it has coiled branched glands called Brunner's gland, the ducts of which open into the base of the crypts. Tunica muscularis consists of a smooth muscle with an inner circular layer and an outer longitudinal layer whereas the external tunic, the tunica serosa is mainly composed of connective tissue layer endowed with blood vessels. The three regions of the small intestine, the duodenum, the jejunum, and the ileum, each have special modifications to the wall to enable each region to better perform its particular

function. In the intestine, digestion occurs in the lumen as well as at the surface of the lining epithelial cells. The smaller breakdown products are then absorbed by the lining epithelial cells called enterocytes (Dellmann and Brown, 1981).

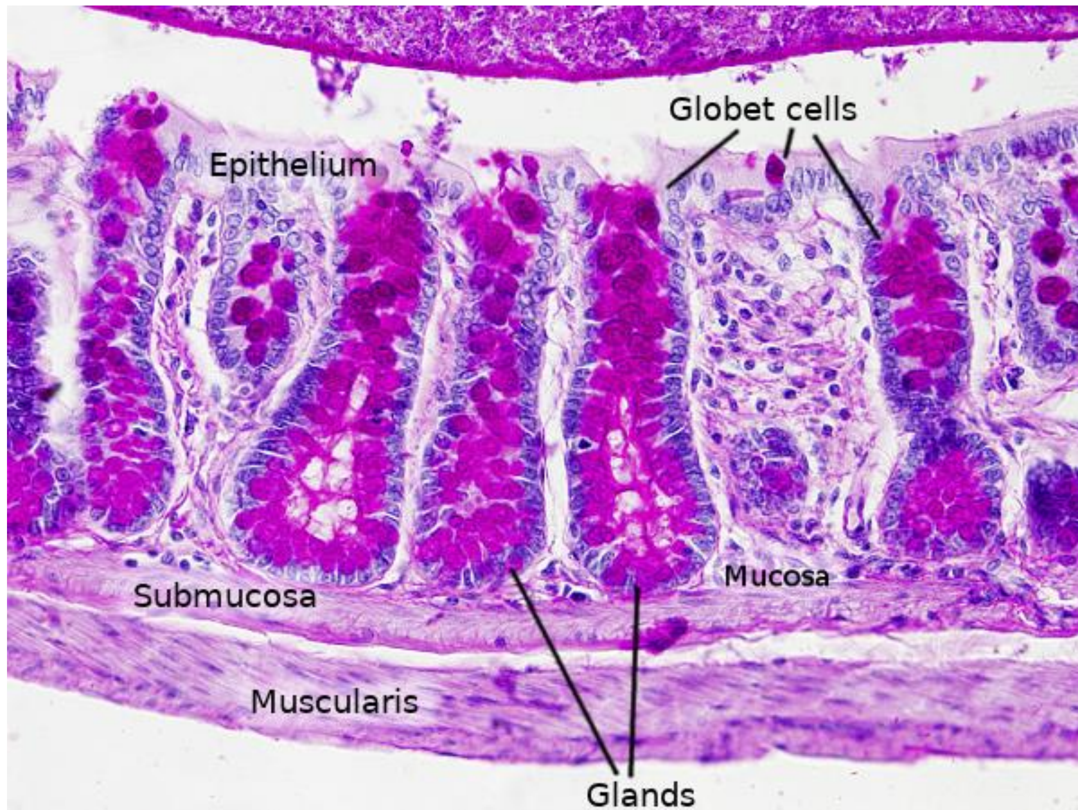

Figure 2: Histologic structure of the small intestine (source: Godwin *et al*., 2016).

2.2.2. Gross and histopathological changes of T. colubriformis infection

The main pathogenic effects of trichostrongylus are caused by stage three (L3), which burrow between the enterocytes of intestinal villi and lead to the formation of intr-epithelial tunnels (Roeber *et al*., 2013). Young nematodes develop in these tunnels for 10–12 days following infection. Gross lesions observed in sheep succumbing to trichostrongylosis are nonspecific and may include cachexia, dehydration, serous atrophy of internal fat depots, and atrophy of skeletal muscle. The intestines are flaccid and the small bowel may contain thin watery green foul smelling feces. The duodenal mucosa may be glistening and pink but superimposed postmortem autolysis may rapidly conceal

the pathological changes. The proximal third of the small intestine (5-7 meters) contains the bulk of the population of the parasites, and a worm count in the small bowel may reveal 15,000-80,000 *Trichostrongylus* in severe clinical infections; subclinical or mild disease is associated with fewer worms (Roy *et al.,* 2004). The migration of young adult worms from the mucosa into the intestinal lumen is associated with extensive damage to the duodenal mucosa and with signs of generalized enteritis, including hemorrhage, edema and plasma protein loss in to the intestinal lumen and subsequent hypoalbuminaemia and hypoproteinaemia (Taylor et al., 2007).

The severity of the *histologic lesion* correlates with the local density of worms. The histologic lesion of *T. colubriformis* is characterized by moderate/ marked villus flattening and atrophy, with focal or widespread loss of superficial epithelium, respectively that may vary considerably in severity. Occasionally stumpy, club-shaped, and fused villi are noted. The cause of villus atrophy is not clear, although it is likely related to the host mucosal immune response against the parasites. Crypts are hyperplastic, dilated, and elongated and there is goblet cell hyperplasia. The lamina propria is populated by a moderately heavy mixed inflammatory cell population, including lymphocytes, plasma cells, eosinophils, mast cells and globule leukocytes which may lead to intestinal anaphylaxis and increased vascular permeability. In most affected gut,inflammatory cells are located at submucosal compartment. In some cases mild or moderate increase of intraepithelial lymphocytes and mild/moderate dilatation of the lymphatic vessels (lymphangiectasia) are observed (Trapani *et al*., 2013). Intestinal anaphylaxis with IgE-induced mast cell degranulation is responsible for changes in the intestine physiology as well as architecture and chemistry of the gut epithelium, including stimulation of fluid, electrolyte, and mucus secretion; smooth muscle contraction; increased epithelial permeability and recruitment of immune cells such as eosinophil or mast cells (Moreau and Chauvin, 2010). Intestinal goblet cell hyperplasia is perhaps the most prominent gut pathology characteristic of infection with nematodes (McKey *et al*., 2017). The intestinal lymph node cells of sheep infected with *T. colubriformis* showed IL5 and IL13 expression which induce infiltration of eosinophils and mucosal mast cell (MMC) hyperplasia (Pernthaner *et al.,* 2005). Increasing in eosinophils in peripheral

blood circulation and tissue is a typical sequence of parasitic infection (Shin *et al*., 2009). Thus, increased levels of IgE in the host infected with different parasites are a well-known process and they have been used as helpful diagnostic criteria. IgE mediates mast cell, eosinophil and basophil degranulation in response to intestinal nematodes and elevation of total IgE serum antibodies has been reported during infection with *T. colubriformis* (Shaw *et al.*, 1998). Significance of IgE in pathology was usually attributed to a type I hypersensitivity reaction and include smooth muscle contraction, increased vascular permeability and local blood flow, and enhanced mucus secretion, while more recent data have shown that the biological importance of IgE includes other mechanisms (Gilfillan and Tkaczyk, 2006). Cell-mediated mechanism of immunopathological lesions in parasitic infections are mainly represented by delayed hypersensitivity reactions. A typical example is the granulomatous reaction around eggs or parasites entrapped in the gut, or intestine (Evering and Weiss, 2006). Recently, it has been shown that several cytokines are responsible for the development and progression of these diseases (Ohsugi, 2007). IL13 plays a role in mucus production and regeneration of intestinal epithelium during parasitic infections and along with IL9 can promote activation of mucosal mast cell (MMC). In sheep, IL5 induces eosinophilia and at the same time promotes IgA production, while IL4 induces switching of induced B cell to produce IgE subclass (McRae *et al*., 2015).

2.2.3. Clinical signs

The disease showed variable depression, inappetence, diarrhea, and reduced weight gain. Although some local mal-absorption of water, electrolytes, and nutrients occurs in the duodenum, it seems unlikely that the absorptive capacity of the remaining small intestine and large bowel would be overwhelmed. Reduced feed intake and increased loss of endogenous nitrogen into the gut because of considerable effusion of plasma protein into the lumen and exfoliation of the intestinal epithelium, likely play some role in the development of diarrhea. In severe trichostrongylosis, compensation for increased catabolism of plasma protein and mucosal epithelial protein is at the expense of anabolic

processes elsewhere in the body; wool and muscle growth are hindered and secondary osteoporosis has been described (Cardia *et al.,* 2011).

3. MATERIALS AND METHODS

3.1. Study Area and period

The study was conducted on purchased sheep and goat in Addis Ababa University College of veterinary medicine and agriculture, Bishoftu, Ethiopia from October 2019 to May 2020. Bishoftu is located 47 km south east of Addis Ababa. Geographically, the area is located at 9°N latitude and 40°E longitude at an altitude of 1850 meters above sea level. It has an annual average rainfall of 892 mm of which 84% is in the long rainy season (June to September). The dry season extends from October to February and the average annual temperature in Bishoftu is 20°C with mean relative humidity of 61.3% (ADARDO, 2007).

3.2. Experimental Design and Groups

A complete randomized study design was used forming four groups. The study at the beginning involved 14 sheep and 14 goats aged between 12 and 18 months but after two weeks of the trial period, one experimental animal from control group of sheep was resigned due to unfit for experimental animal. Thus 13 sheep and 14 goats were included during study period. In goat, seven animals were form infected group whereas the remaining half were kept uninfected controls while in sheep 7 and 6 animals were categorized as infected and uninfected controls respectively. Following purchased, the animals were acclimatized for four weeks during which they are monitored for helminth parasitism and presence of any abnormality that potentially precludes them from the study. All healthy animals were randomized in such a way that each group contains approximately similar mean body weight. All animals were handled and managed according to international guiding principles of animal welfare (Hewson, 2003). They

were provided with grass hay and water adlibitum and supplemented with concentrate mix.

3.3. Experimental Parasites and Method of infection

To generate adequate number of infective larvae for the experimental infection, large number of adult female *T. colubriformis* was collect from the nearby abattoirs and crushed with a mortar and pistil to liberate the eggs. The egg was cultured on parasite egg free animal feces to produce infective stage of larvae (L3). After 14 days of culturing at room temperature under sufficient moisture, the larvae were recovered by the modified Baermann technique (Annex III). After letting the L3 to attain its full infective potential for three weeks at +4 degree, they were drenched to two donor sheep. One month later, up on confirmation of a reasonable fecal egg count set, large volume of fecal sample was collected daily for further culturing to produce adequate number of L3 for the 14 experimental animals. A total of 140,000 L3 were required for the experiment. Recovered L3 after three weeks of maturity was given to each experimental animal at a dose of 10,000L3/animal through oral route according to the method described by Terefe *et al* (2005). Following establishment of a patent infection, all animals were killed after two months of infection for worm recovery and gross and hisopathological sampling and examination.

3.4. Sample Collection and Processing

3.4.1. Blood sample for hematological and biochemical examination

Blood samples were collected from the jugular vein of infected animals. 5 ml of the blood was stored in blood sample bottles containing ethylene di-amine tetra acetic acid (EDTA) for hematological, while another six ml was placed into anticoagulant-free tubes and allowed to clot at room temperature for about 3 hours. The serum samples were later

stored at a temperature of -20°C for biochemical analysis as described by Schalm *et al*. (1986). For hematological and biochemical analysis, blood samples were similarly obtained in 6 sheep and 7 goat served as experimental controls.

The Total red blood cell count:

The total red blood cell (RBC) count was performed in 1:200 dilution of blood in Haym's solution. The blood was taken up to 0.5 marks with RBC diluting pipette and suck the Haym's solution up to mark 101. Mechanically pipette was shacked thoroughly by holding the pipette in between the index finger and thumb. Discarded 1/3 part of contents of pipette and wiped off the tip. The counting chamber and cover slip were cleaned and placed the cover slip in position over the counting chamber by gentle pressure. A drop of blood was added on to the counting chamber by holding the pipette at an angle of 45^{0}
. The cells are settled for 1-2 minutes and total red blood cells in each mm^{3} area are counted under low magnification (10x); and the total red blood cells were determined according to Feldman *et al*. (2000).

Hemoglobin determination:

The hemoglobin (Hb) concentration was evaluated by matching acid hematin solution against a standard colored solution found in Sahl's hemoglobin meter (Dein, 1984). The Sahli method is based on converting haemoglobin to acid haematin (brown colour) and then visually matching its color against a solid glass standard. Diluted hydrochloric acid was mixed into a graduated cylinder with 20µl of blood sample and distilled water was added until the colour of the diluted blood sample matches the glass standard and the dilution was determined based on the Haemoglobin level of the blood sample according to Philippe (2009).

Packed Cell Volume and Blood indices:

Packed Cell Volume (PCV) was measured using microhaematocrit reader from microhaematocrit capillary tubes three fourth filled with blood, sealed and centrifuged at 3000 rpm for 3 minutes. The Mean Corpuscular volume (MCV), and Mean Corpuscular Hemoglobin Concentration (MCHC) were Calculated from total RBC count, PCV and Hb as described by Ibrahim (2013).

Total leucocytes count:

Total leucocyte count (TLC) was also determined by taking the fresh blood up to 0.5 levels in a WBC diluting pipette. Glacial acetic acid is suck up to 11 marks. Mechanically pipette was rotated gently by holding the pipette in between the index finger and thumb. One-third part of contents of pipette was discarded and wiped off the tip. The counting chamber of hemocytometer and cover slip were cleaned. The cover slip was placed over the counting chamber. A drop of blood was added on to the counting chamber by holding the pipette at an angle of 45° and the cells were allowed settle for 2 minutes. Finally total white blood cells in each mm area were identified under low magnification (10x); and the total white blood cell count were determined according to Sharma and Singh (2000).

Differential leukocyte counts:

Blood sample was mixed by rotation before blood smear, prepared with proper blood spread to 2/3rd of slide and air-dried after preparation. Slides were then stained with working wright's stain solution for 3 minutes, washed with tap water, blotted and examined under the microscope for differential leukocyte count with 100x magnification power. The 100 cells were counted for representative sampling of whole blood smear and the percentage of each WBC was determined according to their shape of the nucleus, staining reactions and presence or absence of granules in their cytoplasm. In addition to blood eosinophil percentage, blood eosinophil in ml (Annex II) was also determined by using Fast Read 102 Counting Chambers as indicated by Sharma and Singh (2000).

Serum protein and enzyme analysis:

Collection of 5ml blood for biochemical analysis was carried out from jugular vein and serum was separated after centrifugation at 3,000 rpm for 5 min and stored deep freeze at -20^{0}C until used. The serum total protein and albumin and enzyme activities of aspartate aminotransferase and alkaline phosphatase were measured using an automatic protein and enzyme analyzer machine (AU_protein and vitros 250 for enzymes) as described by Feldman *et al*. (2000).

3.4.2. Necropsy techniques and tissue sampling

Gross pathological examination and lesion characterization:

Animals were killed on day 56 after infection by intravenous barbiturate over dose followed by severing jugular vein. A longitudinal incision at the midline starting from the xiphoid cartilage to the inguinal region was made. The intestines and other abdominal organs were exposed by cutting the abdominal and paracostal muscles along the midline incision. The small intestine of each slaughtered animal was tied at both ends and separated from the other organs with minimal manipulation. The intestinal portions were opened separately and examined for gross pathological lesions before their mucosal surfaces were scraped to remove embedded worms (Boray, 2004). The duodenum, proximal jejunum, distal jejunum and the ileum were demarcated on the basis of their distances from the pylorus for the assessment of the small intestine gross lesion. The duodenum constitute average length of 84.23cm and 72.5cm in sheep and goat respectively from the pylorus, the proximal jejunum extends for 9m from the distal end of the duodenum while the distal jejunum extends for 11m from the distal end of the proximal jejunum in sheep and goat. The ileum extends up to the ileocecal junction with average length of 32.42cm in sheep and 25.85cm in goat (Gutte and Lumbate, 2017). A checklist format (Annex IV) of gross pathological lesions were made as intestinal wall thinning, mucus hyper-secretion, hyperemia, petechial hemorrhage, edema, ulceration,

necrosis, and erosion, thus compared to the different experimental groups and intestinal segments.

Intestinal Worm recovery:

From euthanized animals, the small intestine was sampled and were tied at both ends, cut and placed in labeled tray. The small intestine of each animal was opened longitudinally and the contents were gently washed into collecting jars and filtered through the sieve an aperture of 250µm capable of retaining the adult worms; the contents were then washed into a bucket under running water and the total volume was made up to 2 litres. A duplicate of 200ml was transferred to a labeled plastic container and preserved in 10% formalin. 20ml of the sub-sample was taken onto a petridish, and examined parasites under steromicroscope. The number of worms found in 20ml x 100 gave the total number of worms found in the small intestine as described by Hansen and Perry (1994).

3.4.3. Histopathological techniques

About 4cm square of tissue sample was taken from the lesion and unaffected part of the small intestine and fixed in 10 % buffered formalin for histopathological examination. The preserved tissue samples were trimmed and processed in an automatic tissue processor in different chambers containing different alcohol concentrations (70, 95 and 100%, 100%, 100%), cleared in xylene and embeded in paraffin for preparation into fine blocks (Annex II). Blocks were sectioned at 5 µm, dewaxed, rehydrated (with decreasing concentration of different alcohols) and stained using haematoxyline and eosin (H & E) stain. Then, it was dehydrated again with different increasing concentration of alcohols according to Bancroft and Gamble (2002). Xylene I, II, and II were used to clear alcohols (Annex II). The slides were mounted with Canada balsam and allowed drying before examination under a 40x and 100x magnification power of light microscope.

3.5. Ethical Consideration

According to the ethical standards of Addis Ababa University, there is specific ethics permits were required for the described studies and this was approved by the Addis Ababa University college of veterinary medicine and agriculture animal Research Ethics and Review Committee. The experimental animals were handled properly and killed in humane way without pain and suffering with appropriate euthanasia methods for postmortem examination and sampling techniques.

3.6. Statistical Analysis

Data was recorded, checked and coded on Microsoft Excel spreadsheet (Microsoft Corporation) and R version 3.5.1 statistical software was used for descriptive analysis to describe the data. Chi square (χ^2) test was used to determine lesion frequency between intestinal segments of infected animal groups. The haematological and biochemical means were compared between groups of animals by using the T. test (mean comparison test) at 5% significance level. At 95% confidence level, P values less than or equal to 0.05 are considered as significant as described by Mohammed *et al*. (2013).

4. RESULTS

4.1. Worm Load

In the feces of infected animal group in sheep and goat, *T. colubriformis* eggs were detected beginning at the third week post experimental infection. No eggs were detected in uninfected controls during the entire experimental period. The groups mean adult worm load detected were 3446 and 5016 *T. colubriformis*, which corresponded to the establishment rate of 34.46% and 50.16% of the infective larvae in sheep and goat respectively. In goat, worm burden were ranging from 1980 to 6080 in which six animals recorded more than 5000 adult parasites while in sheep, more than 5000 adult parasites were counted in single animal and ranging from 2000 to 5510 adult worms. Higher mean worm burden with establishment rate was registered in goat than sheep with significance difference ($P<0.05$) (Table 1).

Table 1: Total worm burden (group mean) and worm establishment rate in *T. colubriformis* infected group of sheep and goat

Variables	Experimental animals	
	Sheep infected group (n=7)	**Goat infected group (n=7)**
Male worms	11850	17090
Female worms	12270	18020
Total worm burden	24120	35110
Range	2000 - 5510	1980 - 6080
Group mean	3446	5016
Sex ratios(F/M)	1.03	1.05
Establishment rate (%)	34.46	50.16

4.2. Clinical Responses and Body Weight Changes

At two weeks of post experimental infection in both species of infected animal groups, two sheep and four goats showed alterations in faces, eliminating agglomerated pellets with a "grape bunch" aspect, which had a variable consistency from semi-solid to pasty. The other sheep and goats of the infected group showed normal defecating characteristics throughout the experimental period. This change continued in all individuals of the infected experimental group of animals until the end of the experiment. In contrast, control group of animals had normal fecal consistency. Some infected animals showed signs of depression, discomfort, and minimal decrease body condition. Control animals both sheep and goat have shown a progressive gain in body weight throughout the trial period. On the other hand, animals in the *T. colubriformis* nematode infected sheep, after four week and goat after three week of post exposure showed a slightly reduction in body weight with no significance difference compared to non-infected controls until the end of the trial week (Figure 1). The differences of reduction in live weight between infected and non-infected control sheep and goat were found non-significant (P>0.05).

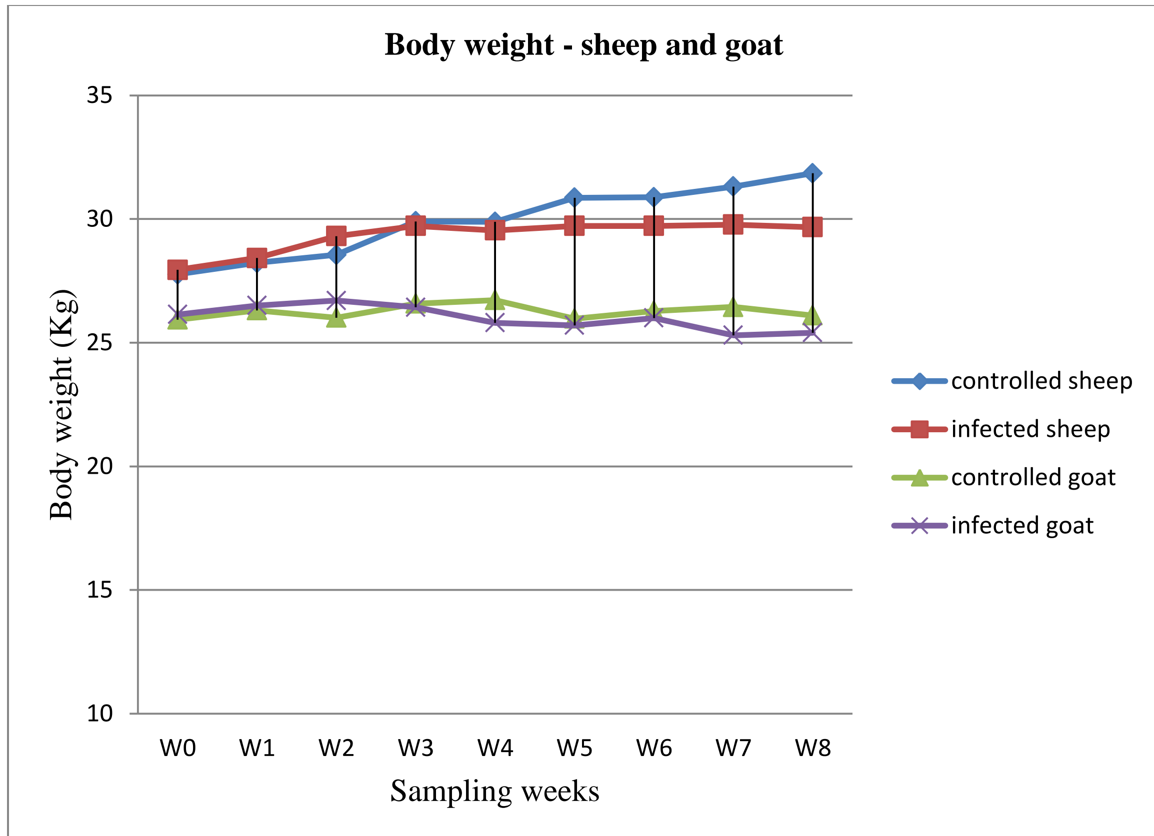

Figure 3: Controlled and infected Body weight in goat and sheep.

4.3. Hematological and biochemical analysis

4.3.1. Hematological examination

Hematological parameters including hemoglobin, hematocrit, red blood cell count, white blood cell count and differential leukocyte count and biochemical indices such as total protein, albumin, AST and ALP were determined. Blood indices were evaluated due to red blood cell counts were made to indicate the evidence of anemia. The mean of PCV and Hb value was decreased in infected group of sheep and goat than uninfected groups with significance difference ($P < 0.05$). PCV and Hb results were lowered from the third weeks of infection up to the six week of the trial period and begin to rise up to the end of the experiment in both species of experimentally *T. colubriformis* infected groups. The overall mean of RBC count was showed lower in experimentally infected sheep and goat

by *T. colubriformis* than non-infected groups and found to be non-significance (figure 4, 5 and 6). Similarly, MCV and MCH mean was decreased in experimentally infected animals except MCHC as compared to control groups in sheep and goat. The overall mean RBC count, PCV, and Hb value for infected sheep were $11.78\pm0.29 \times 10^6/\mu l$, $24.45\pm1.15\%$, and 10.27 ± 0.22 g/dl respectively while the overall means for infected goat were $12.24\pm0.58 \times 10^6/\mu l$ RBCs, $21.60\pm1.78\%$ PCV and 8.32 ± 0.48 g/dl Hb and for the control group, sheep (RBCs $12.50\pm0.26 \times 10^6/\mu l$, PCV $29.42\pm0.38\%$, Hb 12.43 ± 0.36 g/dl) and goat (RBCs $13.65\pm0.41 \times 10^6/\mu l$, PCV $30.03\pm0.60\%$, Hb 11.44 ± 0.21 g/dl) (table 2).

Table 2: Blood parameters (Mean+ SE) of controlled and *T. colubriformis* infected sheep and goat

Parameters	**Sheep**			**Goat**		
	Controlled group(n=6)	**Infected group(n=7)**	**(P value)**	**Controlled group(n=7)**	**Infected group(n=7)**	**(P value)**
Hb (g/dl)	12.43±0.36	10.27±0.22	0.00	11.44±0.21	8.32±0.48	0.00
PCV (%)	29.42±0.38	24.45±1.15	0.00	30.03±0.60	21.60±1.78	0.00
RBC ($X10^6$/ul)	12.50±0.26	11.78±0.29	0.09	13.65±0.41	12.24±0.58	0.07
WBC ($X10^3$/ul)	10.52±0.21	11.99±0.49	0.01	11.03±0.44	12.58±0.52	0.03
MCV(fl)	23.63±0.72	20.77±0.88	0.02	22.14±0.75	17.97±0.76	0.01
MCH(pg)	9.95±0.25	8.76±0.30	0.01	8.45±0.34	6.79±0.14	0.00
MCHC(g/dl)	42.29±1.24	42.75±2.15	0.85	38.27±1.15	38.91±1.72	0.76
WBC differentials						
Neutrophils (%)	40.56±1.54	38.47±1.97	0.41	44.44±2.03	38.82±1.37	0.03
Eosinophils (%)	2.86±0.29	5.2±0.34	0.00	2.47±0.14	6.5±0.84	0.00
Basophils (%)	0.66±0.09	0.37±0.07	0.02	0.42±0.07	0.25±0.06	0.08
Lymphocyte (%)	53.8±1.87	54±1.75	0.19	40.07±2.08	51.77±1.53	0.17
Monocytes (%)	3.6±0.16	3.13±0.38	0.27	3.37±0.24	2.8±0.16	0.06

*Hb: Haemoglobin, PCV: Packed Cell Volume, RBC: Red Blood Cell, WBC: White Blood Cell, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration, g/dl: gram per deciliter, µl: Micro liter, fl: femtoliters, pg: picograms

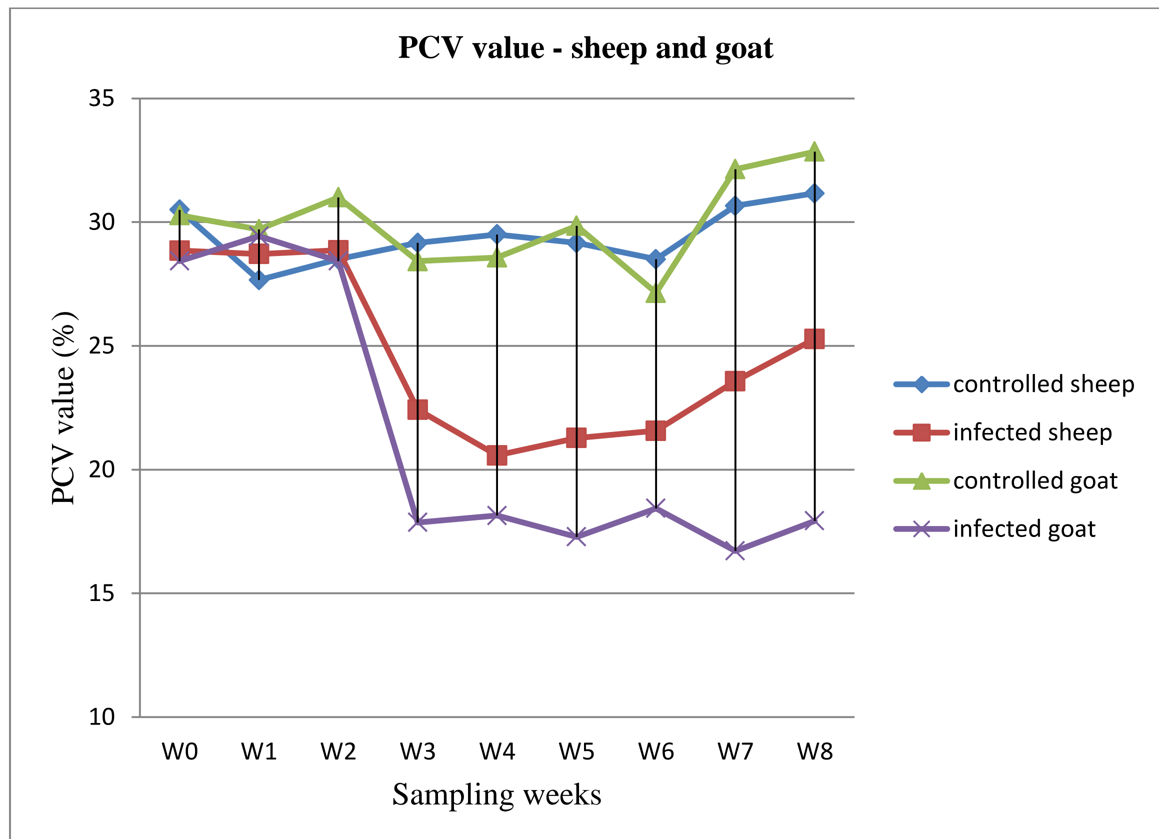

Figure 4: PCV value in *T. colubriformis* infected and controlled sheep and goat.

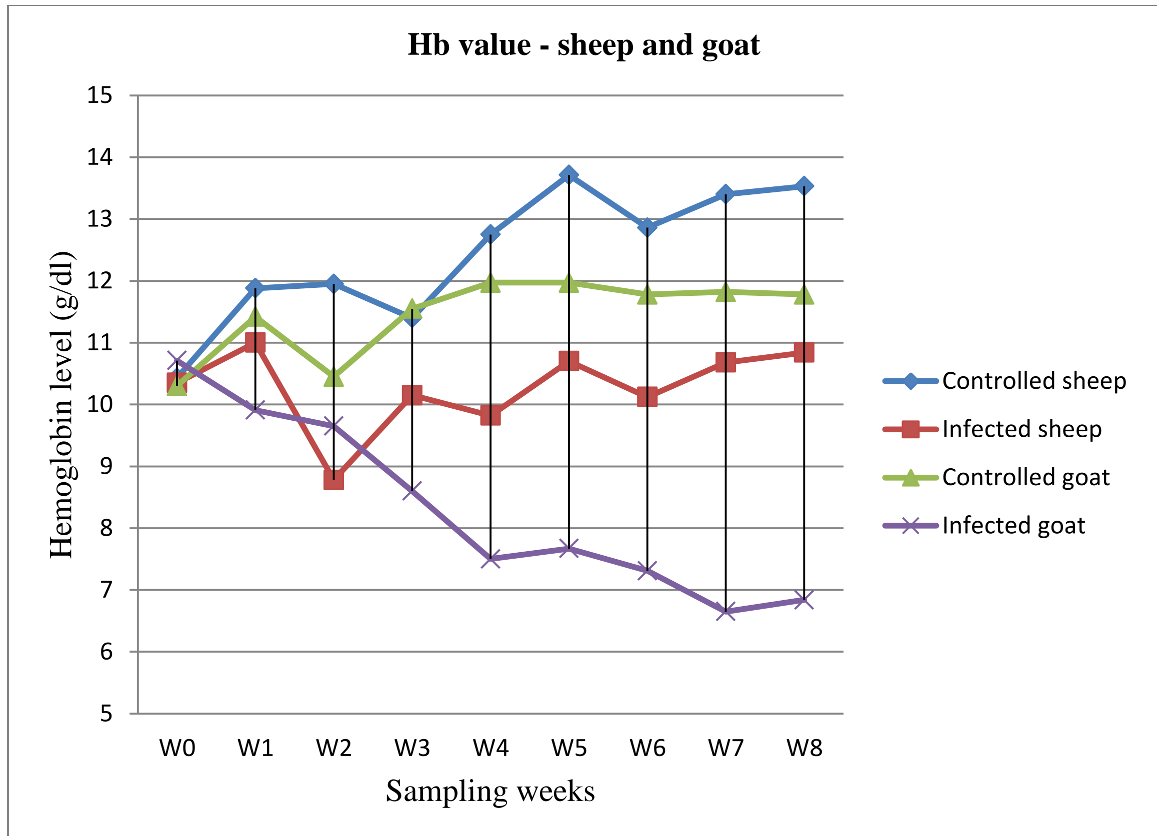

Figure 5: Haemoglobin level registered in *T. colubriformis* infected and controlled sheep and goat.

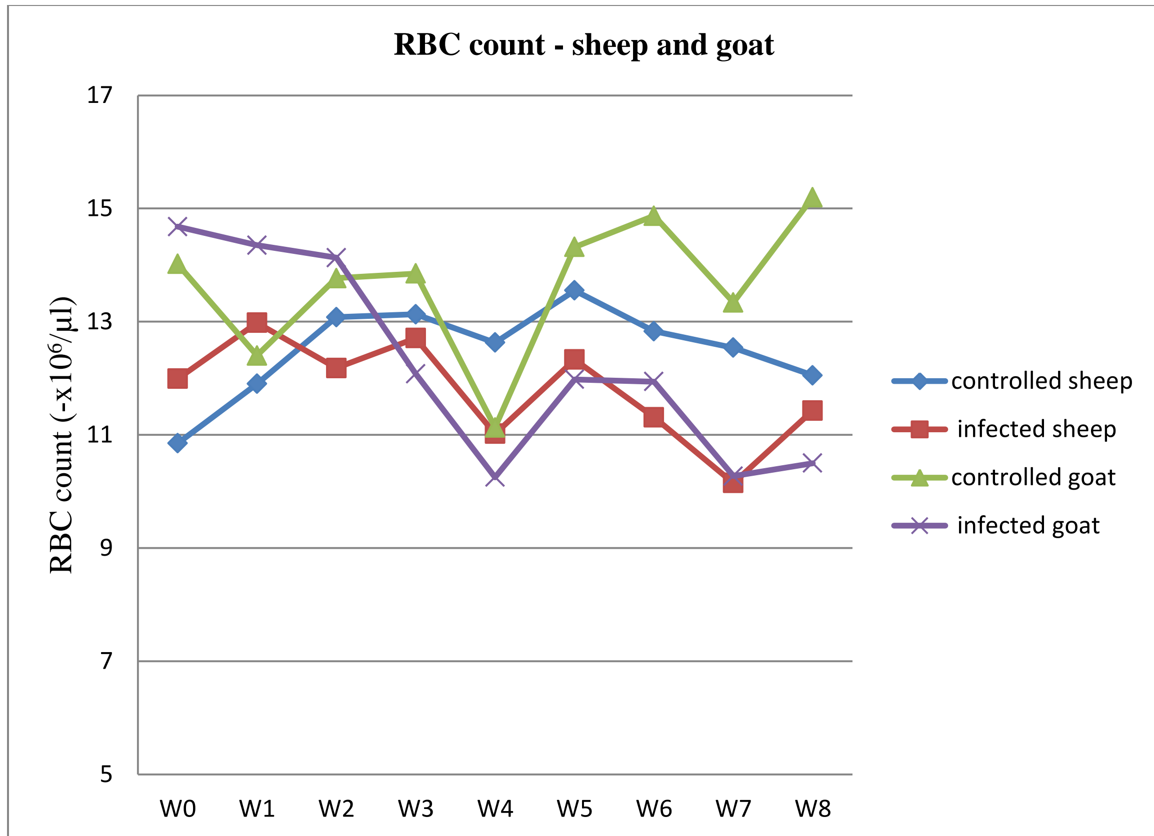

Figure 6: RBC count in *T. colubriformis* infected and controlled goat and sheep.

The total mean leukocyte counts were significantly higher in infected groups of both sheep ($11.99 \pm 0.49 x 10^3/\mu l$) and goat ($12.58 \pm 0.52 x 10^3/\mu l$) as compared to control experimental groups ($10.52 \pm 0.21 x 10^3/\mu l$ sheep and $11.03 \pm 0.44 x 10^3/\mu l$ goat) with a ($P < 0.05$) (Table 2). The mean WBC count was elevated in the first week of *T. colubriformis* infection and peak level was recorded on week three in sheep post infection. The infected goat demonstrated first little peak at week two post infection period and second peak was recorded at week three after post exposure, and then declined until the end of the trial period (figure 7). Similarly the infected sheep was gradually declined up to the end of the experiment after peak at week three of post exposure. Both uninfected control of sheep and goat showed little fluctuating throughout the trial period (figure 7). The infected groups of total mean blood lymphocyte percentage (54±1.75% sheep; 51.77±1.53% goat) without significance difference (P>0.05) were higher than uninfected control (52.3±1.87% lymphocyte sheep; 40.07±2.08% lymphocyte goat) of both animal species.

Others such as blood neutrophils, monocytes and basophils were little fluctuated change along the trial period in both infected and control animals of sheep and goat with no significance difference (Table 2).

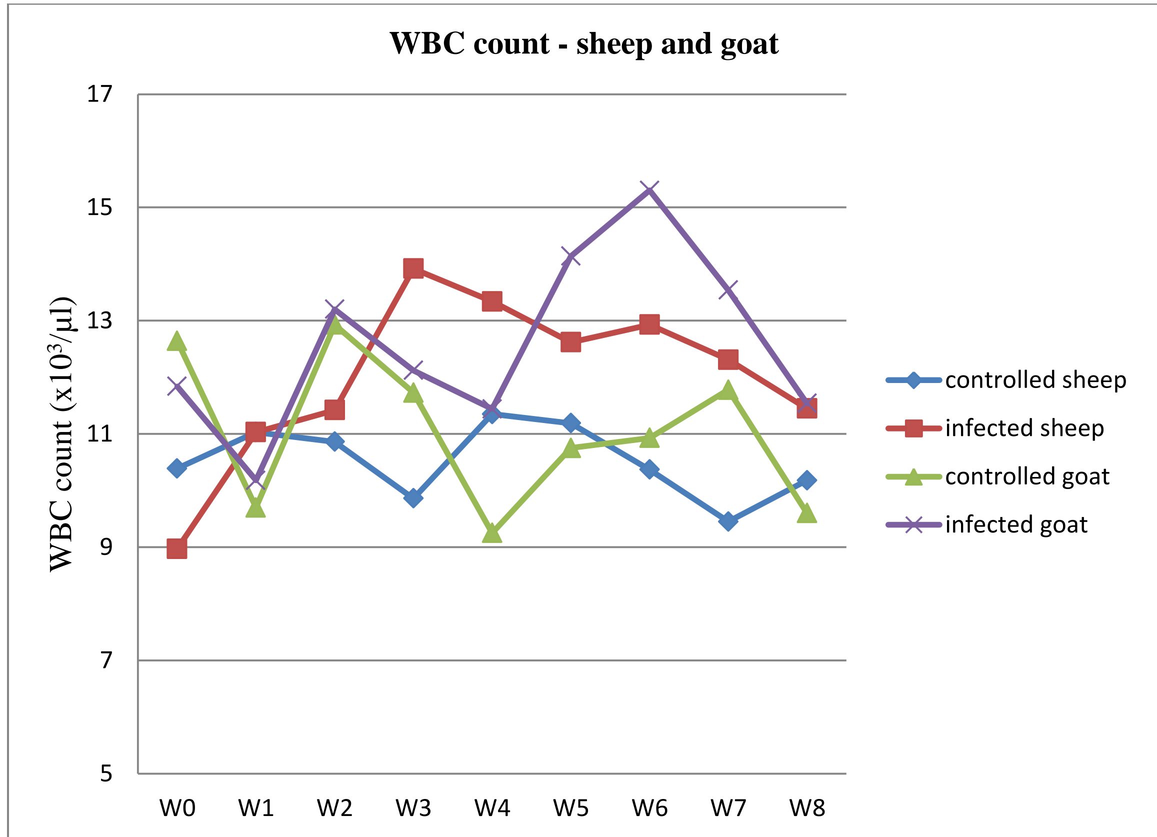

Figure 7: WBC count in *T. colubriformis* infected and controlled sheep and goat.

4.3.2. Blood eosinophil

The weekly mean blood eosinophil number (table 3) was increased significantly ($P<0.05$) in infected group of sheep (week 5 = 72.28×10^4/ml; week 6 = 69.71×10^4/ml) and goat (week 5 = 138.28×10^4/ml; week 6 = 153.42×10^4/ml) than that of uninfected group (week5 = 11×10^4/ml; week 6 = 11.33×10^4/ml) and (week 5 = 37.71×10^4/ml; week 6 = 26.28×10^4/ml) respectively. Peak blood eosinophil numbers were recorded in week 7 for sheep and in week 6 for goat experimentally infected with *T. colubriformis* (figure 8). Towards the end of the experiment, a gradual fall in the level of eosinophil number was

seen in all infected groups of animals. The mean blood eosinophil in all control groups maintained a normal level throughout the experimental weeks. The total mean eosinophil numbers of infected ($53.74 x 10^4$/ml sheep; $61.57 x 10^4$/ml goat) and control ($15.40 x 10^4$/ml sheep; $28.31 x 10^4$/ml goat) were determined. Significantly higher results were found in experimentally infected animals than controls.

Table 3: Weekly blood eosinophil counted (Mean+ SE) in *T. colubriformis* infected and control groups of sheep and goat

Weeks	Sheep			Goat		
	Controlled groups(n=6)	**Infected groups(n=7)**	**(P value)**	**Controlled groups(n=7**	**Infected groups(n=7)**	**(P value)**
Week0	12.66±1.6	9.42±1.83	0.21	7.71±2.11	6.28±2.06	0.63
Week1	20.66±5.64	36.85±7.89	0.13	38.28±15.79	22.85±5.25	0.37
Week2	16.33±4.45	42.57±11.56	0.07	14.85±3.2	18.57±6.91	0.62
Week3	7.33±2.76	17.14±3.69	0.06	13.83±5.45	23.42±7.44	0.31
Week4	5±2.72	44.57±32.1	0.28	35.71±24.14	47.71±13.85	0.67
Week5	11±9.01	72.28±22.97	0.02	37.71±10.51	138.28±31.14	0.00
Week6	11.33±4.86	69.71±21.52	0.02	26.28±12.63	153.42±50.03	0.02
Week7	42±19.82	143.43±80.04	0.27	34.57±14.35	84±27.39	0.13
Week8	12.33±4.42	47.71±12.39	0.03	46±13.23	59.57±14.54	0.50

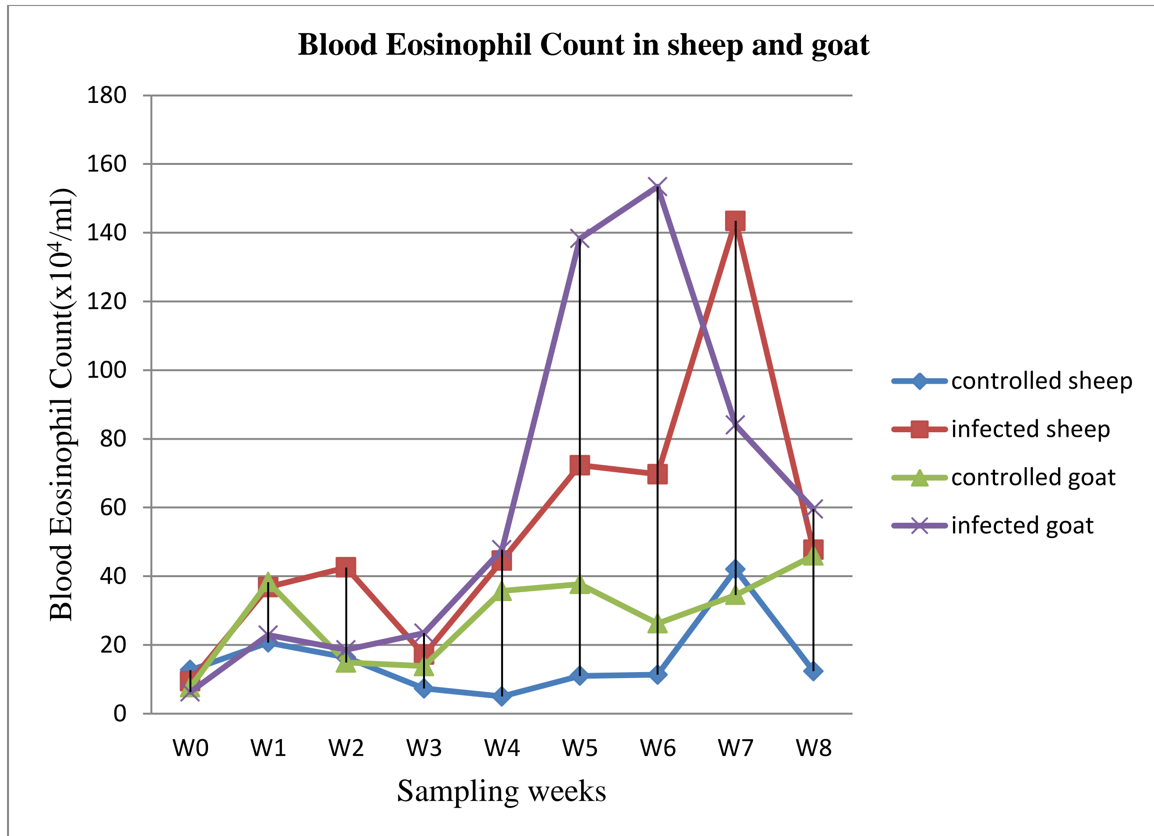

Figure 8: Blood eosinophil count in *T. colubriformis* infected and controlled sheep and goat

4.3.3.. Biochemical findings

The mean values with the serum biochemical parameters, including total protein, albumin, aspartate aminotransferase (AST) and alkaline phosphatase (ALP), in controlled and *T. colubriformis* infected sheep and goats are presented in table 4. From current experiment, *T. colubriformis* infected group had statistically lower (P < 0.05) total mean of serum protein (4.21±0.36 g/dL) and albumin (1.49±0.15 g/dL) than the control group (5.70±0.28 g/dL, total protein; 2.35±0.09 g/dL, albumin) of goat. The values of total albumin serum concentrations resulted the mean (1.87±0.9 g/dL) and statistically significant (P<0.05) while, total protein with mean (4.98±0.27 g/dL) and non-significant (P > 0.05) were decreased in the infected group than control (2.38±0.07 g/dL, albumin;

8.35+2.10 g/dL protein) in sheep. The mean values of both ALP and AST were statistically non-significant between infected and control animals of both sheep and goat.

Table 4: Biochemical indices analysis (Mean+ SE) of *T. colubriformis* challenged and unchallenged sheep and goat

Animals	Bio-chemicals	Controlled groups	Infected groups	P value
Sheep	Total serum protein (g/dl)	8.35±2.10	4.98±0.27	0.06
	Albumin (g/dl)	2.38±0.07	1.87±0.9	0.00
	ALP (iu/l)	151.25±16.96	145.71±11.99	0.78
	AST/GOT(iu/l)	99.6±13.26	105.35±20.04	0.83
Goat	Total serum protein (g/dl)	5.70±0.28	4.21±0.36	0.00
	Albumin (g/dl)	2.35±0.09	1.49±0.15	0.00
	ALP (iu/l)	52.71±4.77	66.54±16.28	0.42
	AST/GOT (iu/l)	59.62±2.74	67.86±3.95	0.11

Note: - g/dl: gram per deciliter, iu/l: international unit per litre, AST: aspartate aminotransferase, GOT: glutamic oxalacetic transaminase, ALP: alkaline phosphatase

Regarding between *T. colubriformis* infected group of sheep and goat, the overall mean PCV, Hb, blood indices, total protein and albumin values were lower in infected goat than infected sheep while total leukocyte and blood eosinophil count were slightly increased in infected group of goat than sheep (table 5). Hemoglobin and albumin values were statistical significant ($P<0.05$).

Table 5: Hematological and biochemical values (Mean+ SE) of *T. colubriformis* infected sheep and goat

Variables	Experimental animals		P value
	Infected sheep	**Infected goat**	
Hb (g/dl)	10.27±0.23	8.32±0.48	0.00
PCV (%)	24.57±1.15	21.60±1.85	0.17
RBC ($x10^6/\mu l$)	11.78±0.29	12.24±0.58	0.50
WBC ($x10^4/\mu l$)	11.98±0.49	12.59±0.52	0.43
Blood eosinophil ($x10^4/ml$)	53.74±13.16	61.57±17.82	0.72
MCV(fl)	20.77±0.88	17.97±0.76	0.03
MCH(pg)	8.76±0.30	6.79±0.14	0.00
MCHC(g/dl)	42.75±2.15	38.93±1.77	0.19
Total protein (g/dl)	4.98±0.27	4.21±0.36	0.09
Albumin (g/dl)	1.87±0.09	1.49±0.15	0.03

4.4. Necropsy of Small Intestine

4.4.1. Gross pathological changes

The postmortem examination of *T. colubriformis* infected sheep and goat revealed gross lesions such as enteritis and mucus hypersecretion, together with petechial hemorrhages, hyperemia, edema and mucosal slough which were especially marked in the duodenum (62.69%) and proximal jejunum (33.33%) in sheep and 47.05% and 45.09% in goat respectively. Frequency of total gross pathological lesions identified were significantly ($P<0.05$) higher in *T. colubriformis* infected goat (40.48%) than sheep (21.43%) (table 7). From a total of gross pathological lesions examined, petechial haemorrhage in sheep 11(40.74%) and goat 17(33.33%) were recorded from small intestine infected with *T. colubriformis*. Macroscopic lesions established (table 6) were higher significantly

($P<0.05$) in the proximal part of small intestine than distal part in both *T. colubriformis* exposed groups of animals. Furthermore, areas with superficial erosions which were rounded or irregular and characterized by breaks in the mucosa, were observed in the small intestine of infected group of sheep and goat.

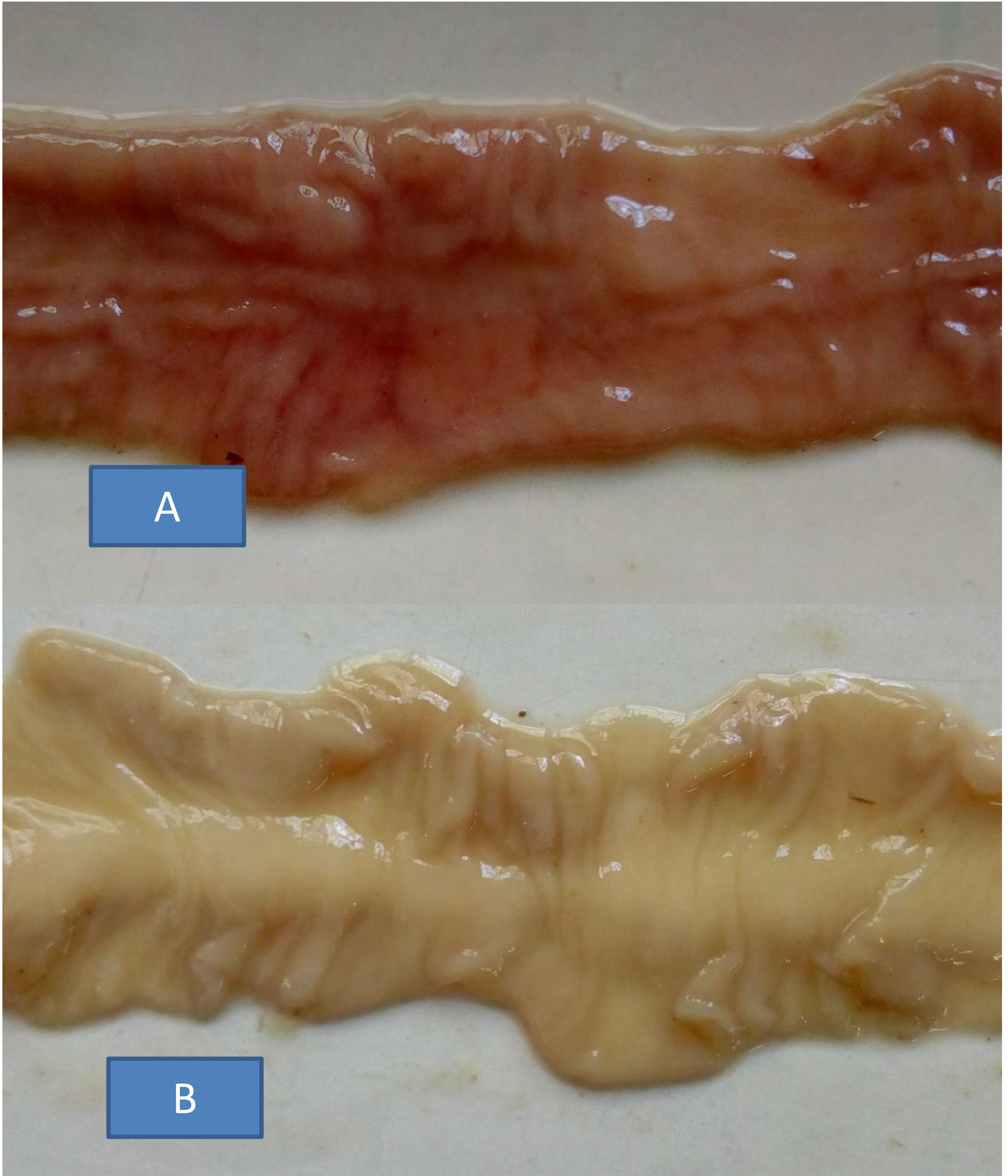

Figure 9: *Trichostrongylus colubriformis* infected and uninfected small intestine (duodenum) of sheep. Gross lesions from small intestine, duodenum of sheep infected

with *T. colubriformis* showing enteritis with hyperemia and petechial hemorrhages (A) and uninfected small intestine (B).

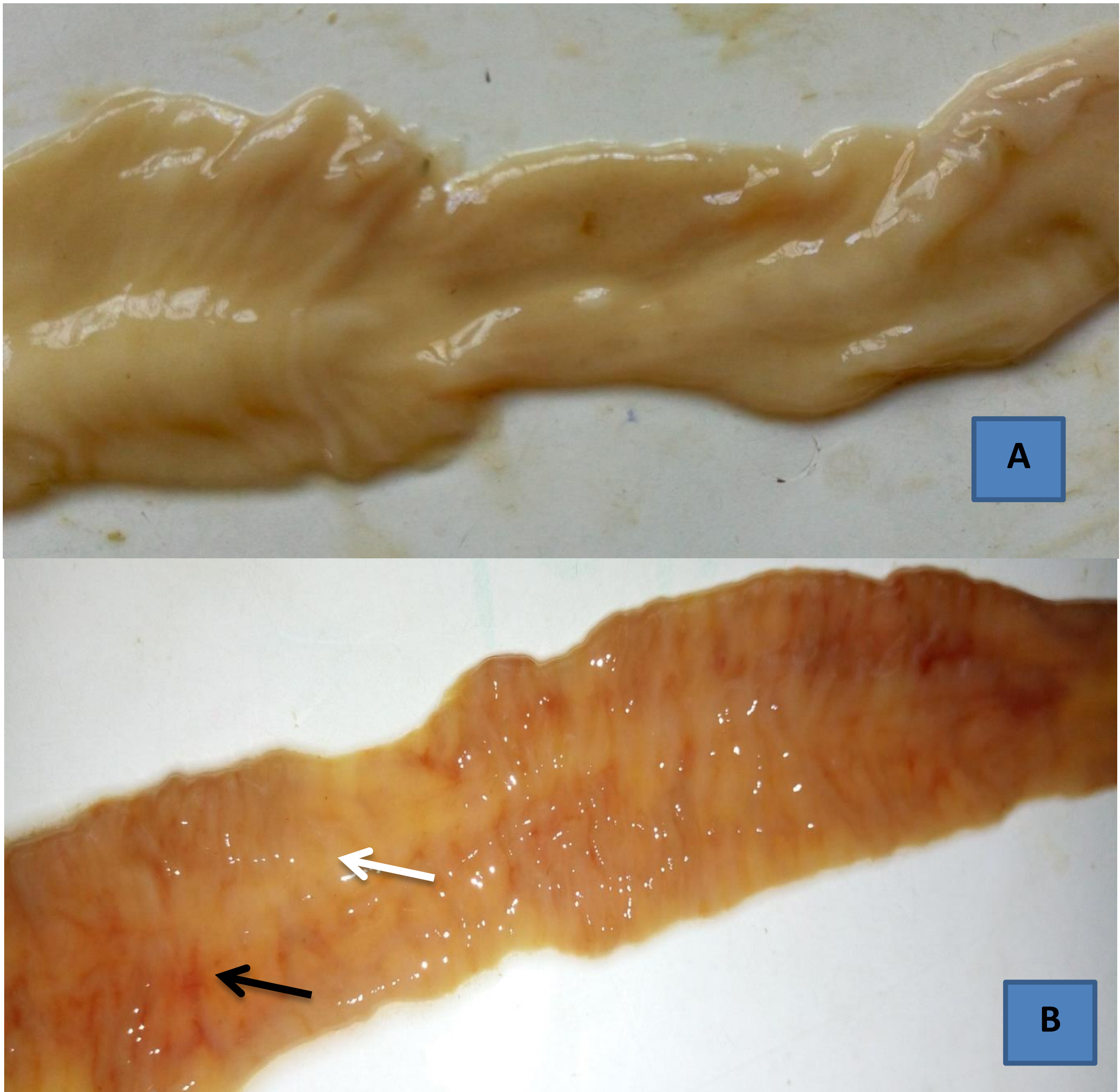

Figure 10: *Trichostrongylus colubriformis* infected goat jejunum**.** Gross lesions from small intestine, proximal jejunum of goat infected with *T. colubriformis* (B) showing enteritis with hemorrhages (black arrow), edema (white arrow) and mild superficial erosions and uninfected small intestine (A)

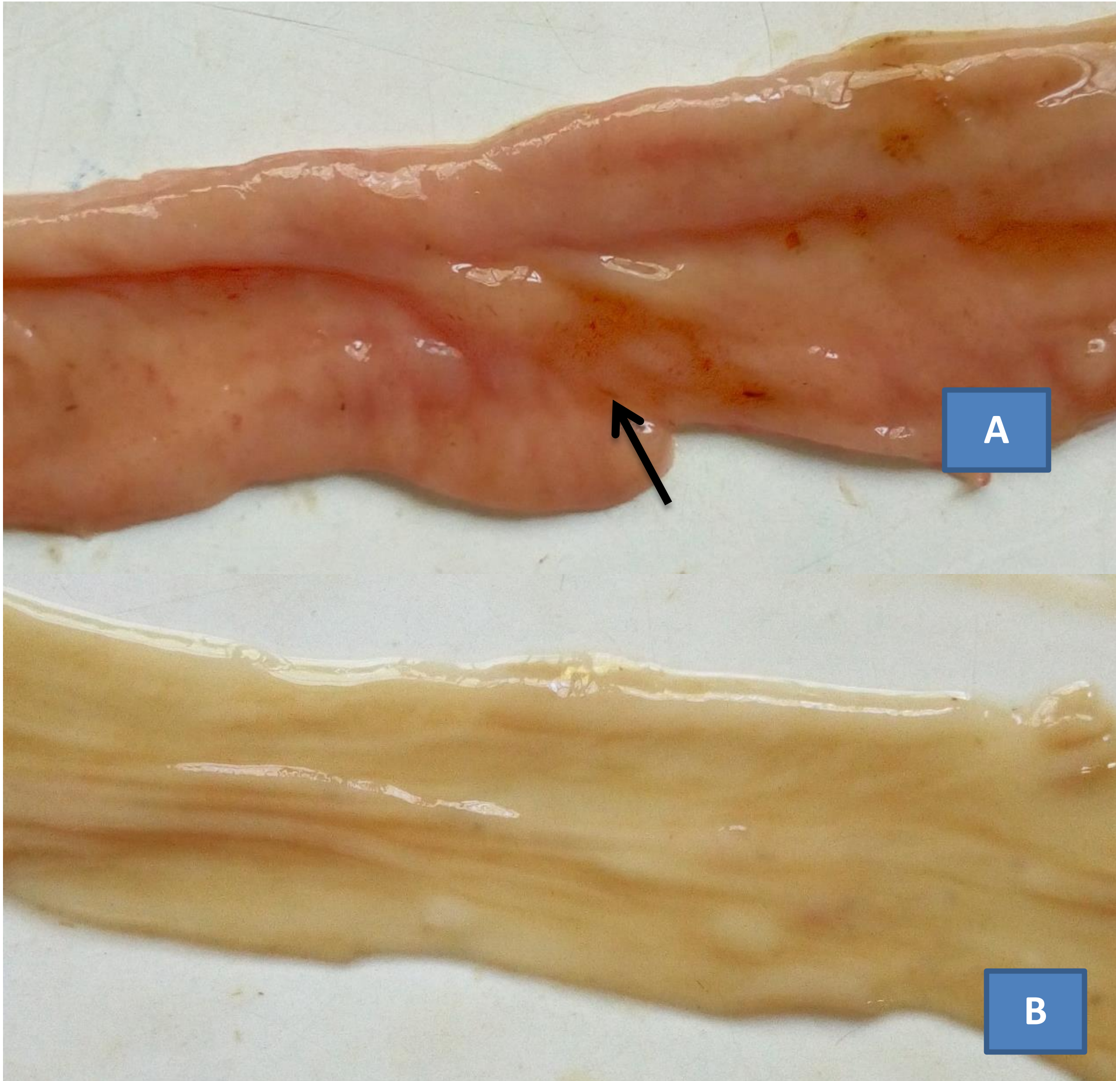

Figure 11: Small intestine of goat infected with *Trichostrongylus colubriformis*. Gross lesions from small intestine, proximal jejunum of sheep infected with *T. colubriformis* (A) showed petechial hemorrhages (arrow) and uninfected small intestine (B)

Table 6: Characterization of gross pathological lesions presented in segments of small intestine of sheep and goat experimentally infected with *T. colubriformis*

Animals	Intestine	Gross lesions					
		Hyperemia	**Petechial Hemorrhage**	**Edema**	**Mucus hyper secretion**	**Mucosal slough**	**Erosion**
Sheep	Duodenum	2	7	3	1	2	2
	P.jejunum	3	3	1	2	-	-
	D.jejunum	-	1	-	-	-	-
	Total (%)	**5(18.52)**	**11(40.74)**	**4(14.8)**	**3(11.11)**	**2(7.4)**	**2(7.4)**
Goat	Duodenum	5	7	5	1	4	2
	P.jejunum	6	7	2	4	4	-
	D.jejunum	1	3	-	-	-	-
	Total (%)	**12(23.52)**	**17(33.33)**	**7(13.7)**	**5(9.8)**	**8(15.69)**	**2(3.92)**

P. jejunum = Proximal jejunum; D. jejunum = Distal jejunum

Table 7: Frequency of gross lesions presented in segments of small intestine of sheep and goat experimentally infected with *T. colubriformis*

Experimental animals	**Intestinal segments**	**lesion frequency**	**Percent (%)**	**X^2(P value)**
Sheep	Duodenum	17	62.96	18.1(0.00)
	Proximal jejunum	9	33.33	
	Distal jejunum	1	3.7	
Goat	Duodenum	24	47.05	25.1(0.00)
	Proximal jejunum	23	45.09	
	Distal jejunum	4	7.84	
	Total gross lesions presented in segments of intestine		**Percent (%)**	**X^2(P value)**
Sheep	27		21.43	10.69(0.00)
Goat	51		40.48	

4.4.2. Histopathological changes

The microscopic lesions (figure 12 - 18) established by *T. colubriformis* were characterized as subtotal villus atrophy, loss of epithelium, petechial hemorrhages, straighten and dilated crypts, erosions, mucosal secretion and inflammatory cells infiltration at the mucosal surface of small intestine were observed in *T. colubriformis* infected goat and sheep jejunum. Total villus atrophy a lesion was also observed in *T. colubriformis* infected goat jejunum. Fused villi and widespread loss of epithelium were seen in experimental infected sheep jejunum. *T. colubriformis* infected sheep duodenum showed mucosal secretion, total villus atrophy and elongated intestinal crypts while in infected goat, duodenum was found total villus atrophy. In both infected experimental animals of duodenum, surface of mucosa was showed leaks and erosions which were sometimes found disrupting the integrity of the epithelium.

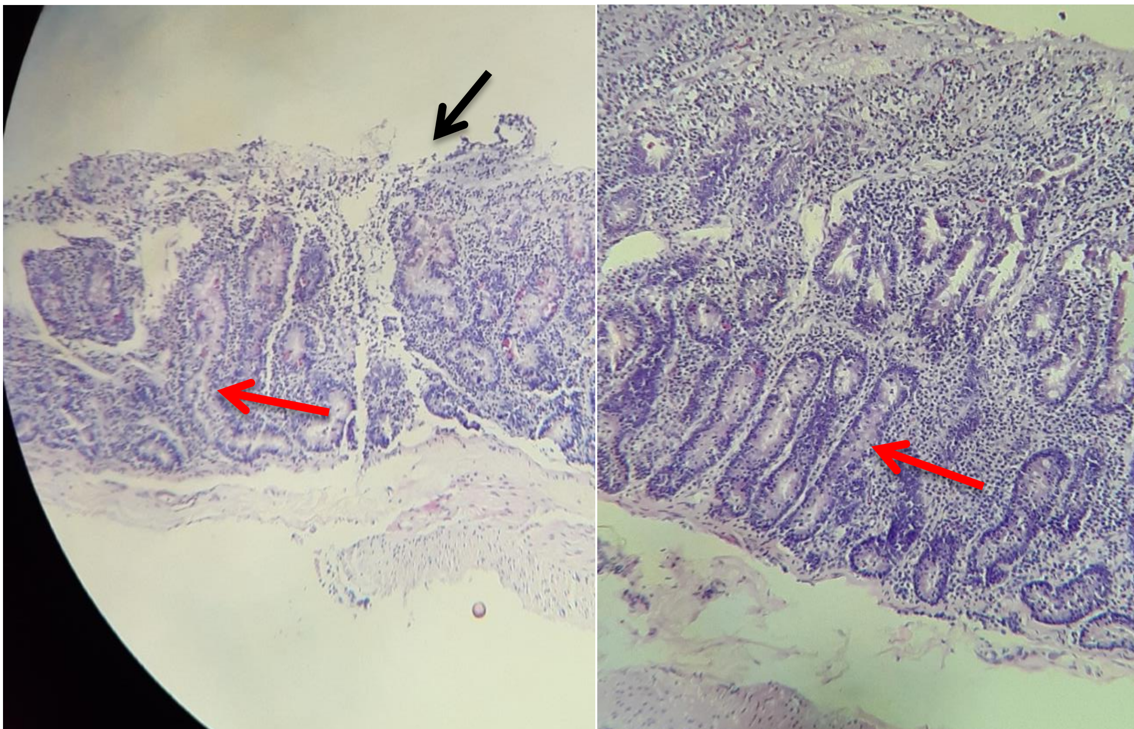

Figure 12: Total atrophy (left) and fused (right) of the villus in the jejunum of goat and sheep experimental infected with *T. colubriformis* respectively*:* Also showed, erosions

(black arrow) elongated intestinal crypts (red arrow), and inflammatory cell infiltration in the lamina propria.

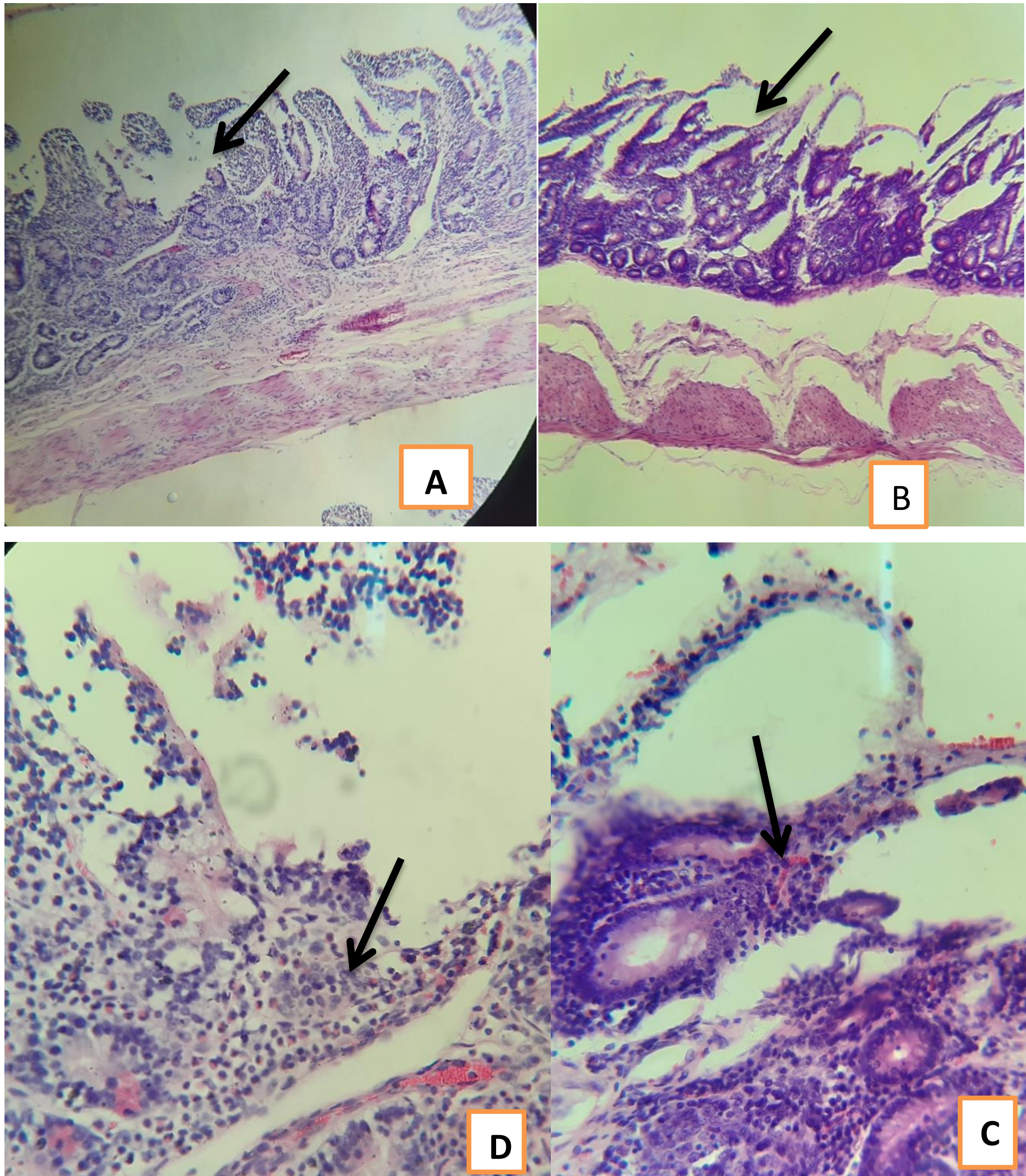

Figure 13: Histological lesions of jejunum in goat (A&D) and sheep (B&C) experimental infected with *T. colubriformis*: Showed subtotal total villus atrophy, erosion (arrow), loss of enterocytes, inflammatory cell infiltration, mucosal secretion and

hemorrhages at the mucosal surface and similarly in sheep with parallel, dilated crypt, erosion (arrow), hemorrhages and infiltration of inflammatory cells in the mucosa.

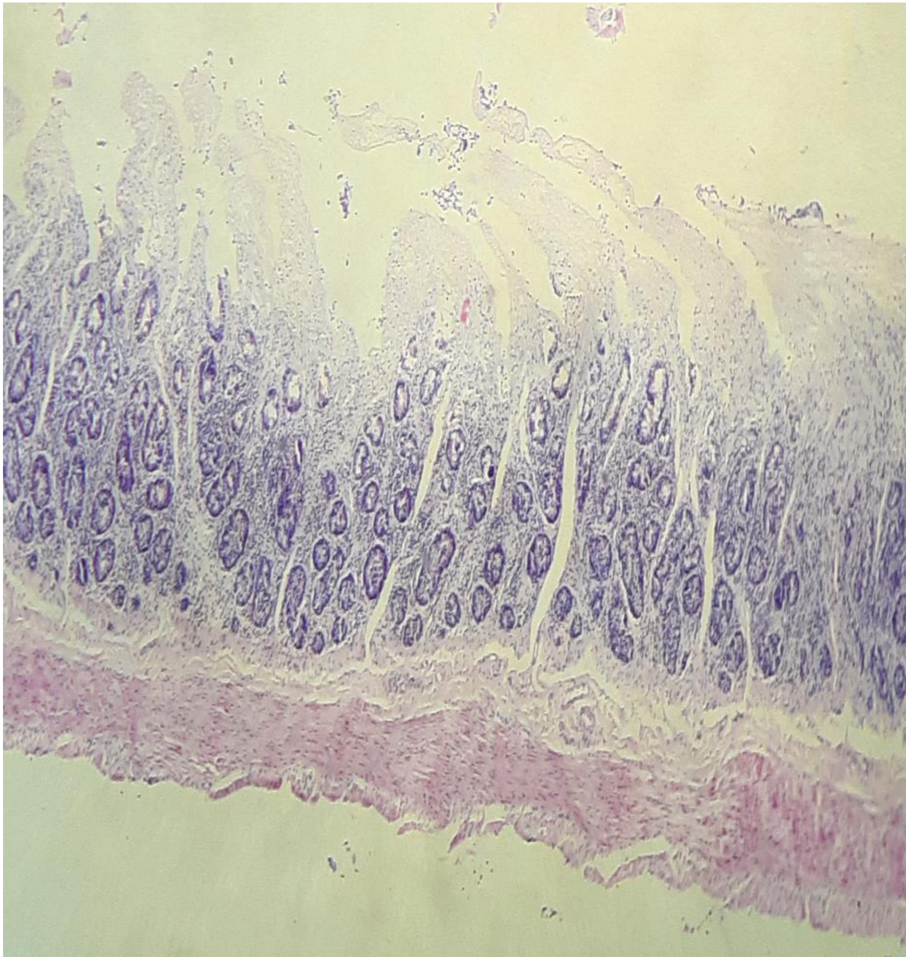

Figure 14: Histological section of mucosa, uninfected sheep small intestine (jejunum)**:** Showed tall villi and the intestinal crypts are unaffected

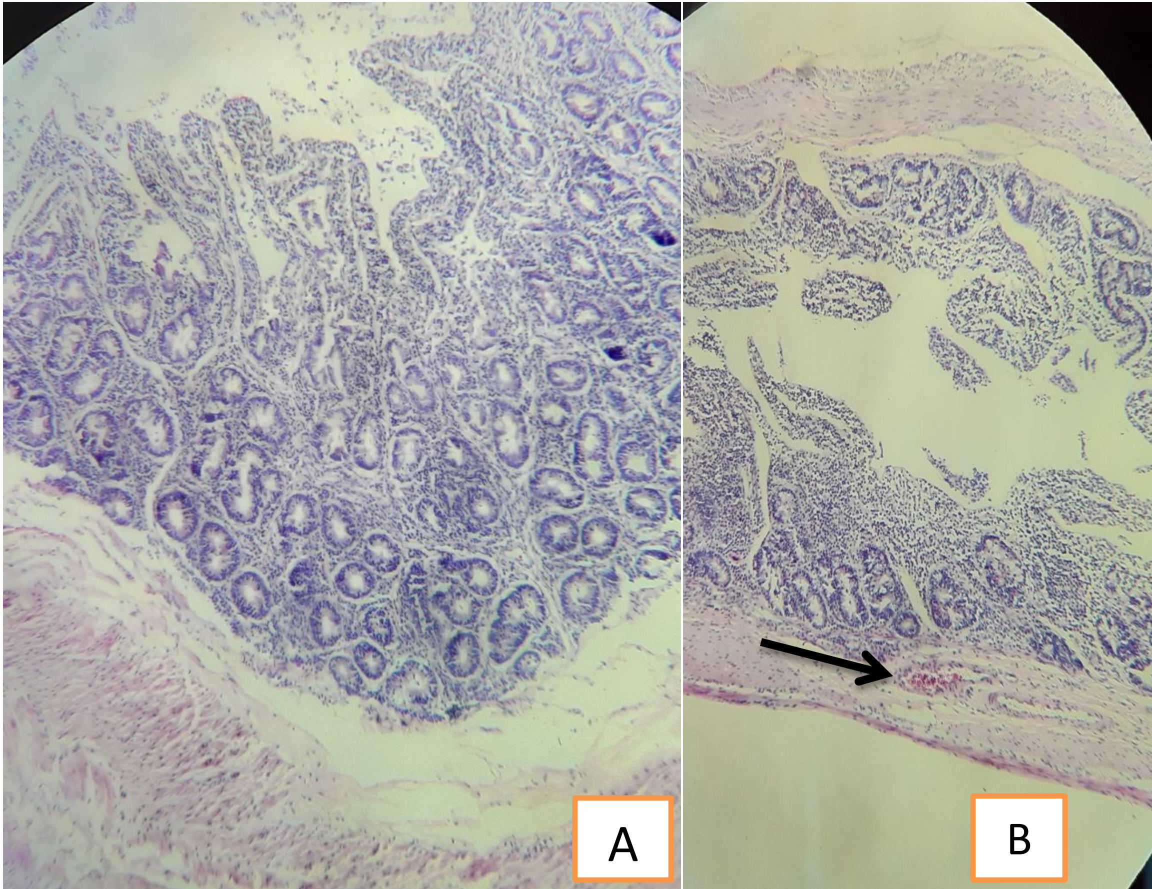

Figure 15: Section of mucosa, uninfected (A) and infected (B) goat small intestine (jejunum): Uninfected jejunum showed tall villi and the intestinal crypts are not elongated and dilated while *T. colubriformis* infected jejunum showed total villous atrophy and hemorrhage in sub mucosal layer (arrow)

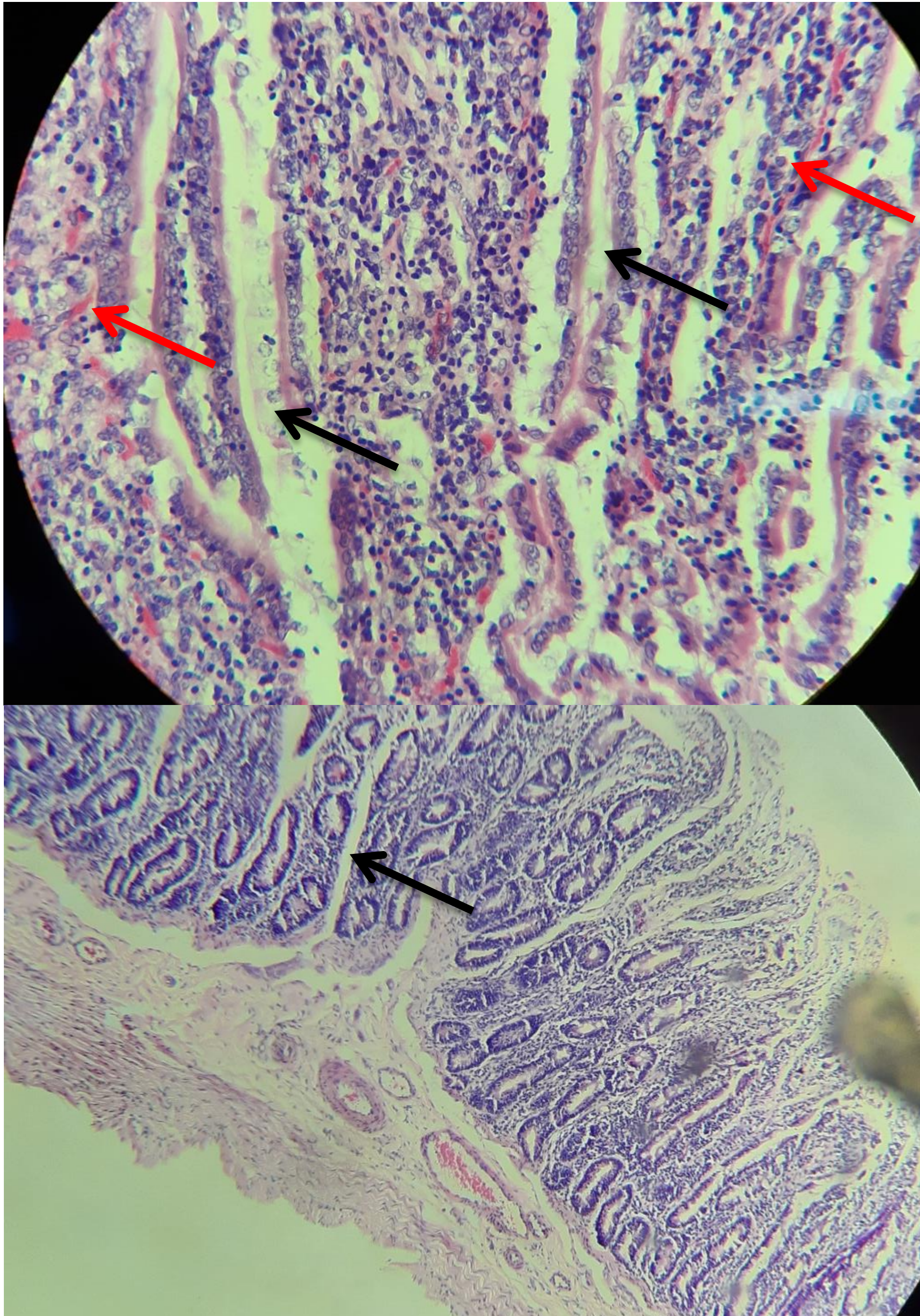

Figure 16: Histological section of intestinal mucosa, infected goat jejunum**:** Showed elongated, dilated and opened intestinal crypts (black arrow), enterocyte loss, sub villus atrophy and hemorrhages (red arrow).

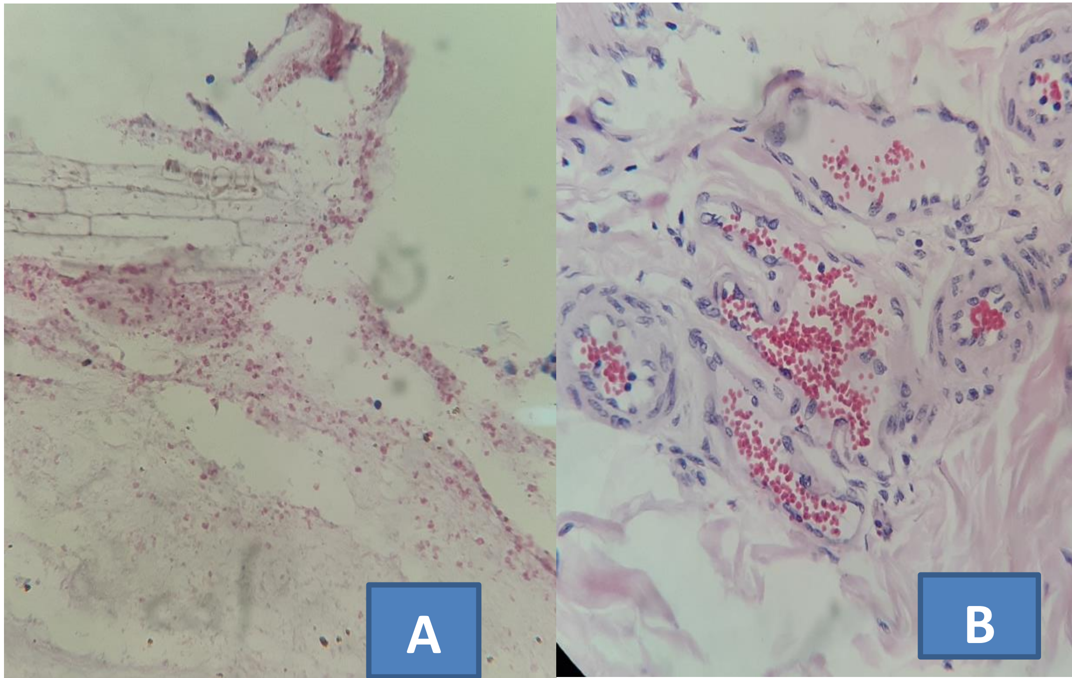

Figure 17: Histologically, hemorrhages at the surface of mucosa (A) and in sub mucosa (B) of *T. colubriformis* infected goat jejunum

5. DISCUSSION

This study was carried out to assess hematological, serum biochemical and pathological alterations in indigenous Ethiopian highland sheep and goats experimentally infected with *T. colubriformis*. The artificial infection of *T. colubriformis* in sheep and goat showed an influence on values of hematological and biochemical parameters and intestinal pathological conditions. The depression and decrease in appetite was minimal and not the main disorder resulted from *T. colubriformis* infected sheep and goat. This may associated with the number of infective larvae given during experimental study period. According to the previous reports of Steel *et al.* (1980) and Symons (1983), severe consumption disorders presented in young lambs were as a result of infected with a large number of *T. colubriformis* larvae. The reduced appetite of animals infected with this nematode may be due to an increase in the plasma concentration of cholecystokinin (CCK) hormone which stimulated and elevated in *T. colubriformis* infected sheep *(*Symons and Hennessy, 1981).

The hematological results revealed reduction in PCV, hemoglobin and RBC values with significant difference in infected animals than non – infected control group of sheep and goat. This is probably as a result of hemorrhagic lesions in intestinal tissues, as observed from histological analyses and tumour necrosis factor-α (TNF-α) has also been implicated in suppressing haematopoietic progenitors during inflammation, resulting in decreased erythrocyte production and decreased erythrocyte survival (Boulter and Hall, 2000). Pathological conditions and laboratory results may differ depending on the factors such as the intensity of infection and species of nematodes. Thus, changes in PCV and RBC values are more common in parasitism by species of nematodes, such as *H. contortus* (Shakya *et al*., 2009). The reports of Roy *et al.* (2004) also described that, the severity of pathological establishment was higher when 30,000 infective larvae of *T. colubriformis* were administered than 15, 000 larvae.

The overall hemoglobin and total RBC count observed for both infected and control goat and sheep were within the normal reference interval stated in veterinary medicine, a textbook of the disease of cattle, sheep, goat, pig and horse (Radostits *et al*., 2007) suggesting the absence of development of significant anemia. However, the infected sheep and goat group were registered lower PCV, hemoglobin and total RBC count than uninfected controls. These results (29.42$\pm$0.38, control sheep; 24.45$\pm$1.15, infected sheep) were in agreement with the report of Cardia *et al.* (2011) as the PCV mean of the infected group (29.80 $\pm$ 0.49) was significantly lower ($P < 0.05$) than the PCV of the control group (32.70 $\pm$ 0.79) in lambs with artificial *T. colubriformis* infections. Other researchers such as Horton *et al.* (1977) also reported that mild increased in Hb, erythrocyte and haematocrit values in non-infested lambs and lowest for infected lambs which are consistent with this finding. In the present finding, even Radostits *et al*. (2007) stated that haematological differences were affected by host and species factors, lower PCV and hemoglobin values were recorded in *T. colubriformis* infected goats than sheep.

The Mean Corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) blood indices were decreased except Mean Corpuscular Hemoglobin Concentration (MCHC) which was unchanged between infected and healthy animal groups. The present findings of blood indices were supported by Horton *et al.* (1977). In infected experimental groups of the current finding, mild decreases were observed in MCV than non-infected groups which was disagrees with the report of Horton *et al.* (1977) but it was in the normal reference level indicated by Radostits *et al.* (2007). Reduction in MCV may be due to decrease in PCV level may be attributed to the dilution of blood (Schetters *et al.,* 2009). Blood parameter alterations can be also associated with inhibition of mineral and vitamin absorption such as vitamin E, selenium and iron from the intestinal mucosa as a result of *T. colubriformis* infection. Horak *et al.* (1968) have been reported hematological changes were observed in lambs infected with *T. colubriformis* and Hoekstra (1974) was described that selenium has been shown to have a specific enzyme function in sheep red blood cells. Horton *et al.* (1997) also added that Hb, erythrocyte and PCV values were lowest for *T. colubriformis* infected lambs receiving vitamin E and Se than uninfected

lambs feeding with vitamin E and Selenium but MCV was not affected by either *T. colubriformis* infection or supplementation of Vitamin E and Selenium.

The mean of total leukocyte count revealed a significantly increased in both infected group of sheep and goat than non - infected controls. The observed leukocytosis in infected sheep in the current study is consistent with the finding of Horton *et al*. (1977), leucocyte counts were higher in infested lambs than that of the non-infested groups. According to the reports of Furlanello *et al.* (2005), leukocytosis occurred due to maturation of lymphocyte and neutrophil. Intestinal nematode damage intestinal mucosa which leads to activation of macrophages that release pro-inflammatory cytokines (Th2 associated cytokines), including interlukin (IL4, IL5) and tumor necrosis factor (TNF) and interlukin-4 and 5 causes the proliferation of lymphocytes. The later TNF also important for activating of blood momonuclear cells such as lymphocytes which are responsible for lymphocytosis **(**Duque and Descoteaux, 2014). The differential leukocyte counts were found normal in all experimental animals except slight increase in lymphocyte percentage of infected groups of sheep and goat.

The mean blood eosinophil count was increased significantly in infected group of sheep and goat than control group. Similar results were found in the reports of Cardia *et al.* (2011). The blood eosinophil count between *T. colubriformis* infected group of sheep and goat of the present study was non-significant. The eosinophillia may serve as an indicator of host's responsiveness to *T. colubriformis* infection (Dawkins *et al*., 1989). According to the finding of Dawkins *et al.* (1989) noted marked eosinophilia in the high responder lambs following challenge with *T. colubriformis.* Moreover, ovine gastrointestinal nematode infections cause a lot of factors that induce migration of blood eosinophils according to Wildblood *et al.* (2005). Evidences from different studies suggested that eosinophils have been viewed as an important indicator of helminth infection and pathogenesis (Nickdel *et al.,* 2001). The registered eosinophilia was as a result of animal's sensitivity to the foreign protein of a parasite which may be a part of an immune phenomenon (Feldman *et al*., 2000).

The major alterations were also occurred in the mean values of total serum protein and albumin concentration in both infected groups of experimental animals. Greater total serum protein and albumin loss were observed in *T. colubriformis* infected goat than sheep. This is probably due to decrease in protein absorption and albumin loss into the intestinal lumen through the lesions developed by the worm load in the intestinal epithelium as evidenced by increased worm burden was registered in infected group of goat than sheep. Lower serum concentration of albumin and protein values were also recorded in infected animals of sheep and goat than non-infected controls. Related results with the present study were reported by Cardia *et al.* (2011). Horton (1977) also evidenced that total serum protein and albumin concentrations were depressed by about 14% ($P < 0,001$) in *T. colubriformis* infested lambs as compared to non-infested animals. In the present study, the infected group of sheep and goat presented the lowest albumin serum concentration than uninfected controls at the end of experimental period. Cardia *et al.* (2011) observed that mean values of albumin serum concentrations were significantly lower in the infected group than uninfected control lambs which support the current results. The rejection of *T. colubriformis* incoming larvae by immune animal is accompanied by an intestinal inflammatory response involving the secretion of biogenic amines with a concurrent plasma albumin loss. This is the major factor responsible for the cause of decreased blood protein and albumin level in sheep infected with *T. colubriformis* (Steel *et al*., 1980). The findings of the present study regarding the activities of aspartate aminotransferase and alkaline phosphatase were non-significant ($P<0.05$) in infected and control groups of sheep and goat. The current results were inconsistent with the reports of Horton *et al.* (1977) that aspartate aminotransferase values were significantly ($P<0.001$) higher in lambs infected with *T. colubriformis* than control. This disagreement between previous and present report may be associated to the experimental infective *T. colubriformis* larvae doses which were 100,000 in the previous while 10,000 were in the current study. Kumar *et al.* (2015) also described in their clinicopathological studies of gastrointestinal tract disorders in sheep with parasitic infection that the activity of AST and ALP were significantly increased in GIT parasitic infected sheep which disagrees with present finding. According to Kumar *et al.* (2015),

specific hepatic functions are greatly affected by a wide variety of the pathological condition of extra hepatic origin especially gastrointestinal origin. These enzymes have their function and greatest concentration within the cell, thus the increase in enzymatic activities reflect cellular abnormalities which directly related to hepatocytes damage, intestinal pathological lesions and cardiac infarction (Purohit *et al*., 2003) which were not evidenced in the present finding. This difference between the previous and current reports in the development of pathological changes may be associated with the species and burden of parasites.

The gross and microscopic intestinal pathology and their characteristics which are consistent with the injuries caused by *T. colubriformis* infection have been previously studied in small ruminants but no more comparable recent data between sheep and goat are available. The predominant characteristics of gross intestinal *T. colubriformis* induced lesions in sheep and goat of the present study includes enteritis and mucus hypersecretion, together with edema, hyperemia, petechial hemorrhages and mucosal slough were markedly observed in the duodenum and proximal jejunum. Beveridge *et al.* (1989) and Roy *et al.* (2004) results were reported that 37 – 45% of *T. colubriformis* infection in sheep occurred primarily in the proximal region of small intestine two metres from the pylorus were in agreement with the present study. The overall macroscopic lesion frequencies of percentages were greater in infected group of goat than sheep. This result may relate with the number of worm burden found in the site of infection, as it was greater in goat than sheep infected animals. Trapani *et al.* (2013) reported that the severity of the lesion correlates with the local density of worms. According to the reports of Abebe and Esayas (2001) in arid and semiarid zones of eastern Ethiopia, the prevalence of *T. colubriformis* in goat (89.90%) at post-mortem examination was greater than in sheep (87.15%) which were supported with the present study of worm establishment rate in goat (50.16%) and sheep (34.46%). Williams *et al*. (2010) also reported that rams were able to resist the *T. colubriformis* larval challenge effectively as registered the small total worm count were evidenced the present finding of low worm burden in sheep than goat. This worm burden and pathological difference between *T.*

colubriformis infected sheep and goat may be associated with factors such as species and breed resistance of the host.

The microscopic lesions observed in the duodenum and proximal jejunum of *T. colubriformis* infected sheep that is villus atrophy and crypt hyperplasia of the current study were less severe than the total atrophy described by Barker (1975a) in clinical trichostrongylosis, but were comparable to previous descriptions in sheep infected at a subclinical level with *T. colubriformis* (Beveridge *et al,* 1989; Roy *et al.,* 1996) and in experimental infections of sheep with *T. colubriformis* on nematode distributions, numbers and on pathological changes (Roy *et al*., 2004). Trapani *et al*. (2013) were observed similar degree of villus atrophy with the present finding in goats naturally infected by *T. colubriformis.* Fused villi with widespread loss of intestinal epithelium were identified in the present study in experimental *T. colubriformis* infected goat which were consistent with the reports of Trapani *et al*. (2013) in natural *T. colubriformis* infected goat. Erosions of intestinal epithelium of the current findings were also comparable with the results of Barker (1975a) in his study of intestinal pathology associated with *T. colubriformis* infection in sheep but less severe than the current finding of experimental *T. colubriformis* infection in sheep. Beveridge *et al. (*1989) also added related reports with the present study that sloughed epithelial cells are present in the lumen as well as significant number of inflammatory cells. The severity of pathological changes can be associated with the total numbers of larvae administered and the total numbers of worms which establish at site of infection. The changes may be due to a mechanical effect of the parasite or due to chemical secretions from the worms.

Similar to the reported by Trapani *et al*. (2013) and Paciello *et al.* (2004) in goat, this study indicated that the immune response to *T. colubriformis* infection in sheep and goats was characterized by an increase rate of infiltrating inflammatory cell in the intestinal mucosa. Trapani *et al*. (2013) were also noted that, in the lamina propria there were inflammatory cells consisting of lymphocytes, plasma cells, eosinophils, macrophages and globule leukocytes in *T. colubriformis* naturally infected goats. In most affected gut, inflammatory cells were located at submucosal compartment. In some cases mild or

moderate increase of intraepithelial lymphocytes and moderate dilatation of the lymphatic vessels were observed (Trapani *et al*., 2013). The precise role of infiltrating inflammatory populations during infection with the parasite and the mechanisms by which these cells may influence the sheep and goat immune response are important issues that remain to be elucidated.

6. CONCLUSION AND RECOMMENDATIONS

The current study was described the effect of *T. colubriformis* experimental infection on hematological parameters, biochemical indices and pathological changes in the small intestine of sheep and goat. The study ensued, *T. colubriformis* adult worm burden and its pathological effect was greater in goat than sheep. The study showed that experimental infection of *T. colubriformis* were affected blood parameters, biochemical indices and caused pathological alterations of small intestine in sheep and goat. Thus, in the present study *T. colubriformis* infection was not a significant cause of anemia in sheep and goats even though slight reduction of blood parameters were observed in infected animals than controls. Generally, this experimental study concluded that pathological, hematological and biochemical effect of *T. colubriformis* infection was greater in goat than sheep even statistically it was non-significant. Thus, the recommendations forwarded were:

- Attention should be given to further pathological investigation of *T. colubriformis* infection in sheep and goat small intestine.
- Additional studies that addressing these comparative aspects of *T. colubriformis* infection in sheep and goats are required.
- Improving host immunity against *T. colubriformis* by foranging and pasture management strategies to reduce worm burden.
- Providing appropriate treatment: Deworming with anthelmintic, correction of blood parameter alterations that can leads to anemia through mineral and vitamin supplements are recommended.

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8. ANNEXES

Annex I. Hematological Analysis Procedures

Hemoglobin Determination (Ibrahim, 2013)

- Take 0.1N HCL (1%) into centeral graduated tube up to mark
- Suck the blood exactly up to mark 20 (20µl) with the help of sahlis pipette
- Transfer the blood from pipette to central graduated tube of the hemometer
- Mix it well with the help of stirrer or rod and allow it to react for two minutes
- Make up with distilled water by adding drop by drop until the color matches with Standard comparator tube and mix well.
- When the color matches take out and record the values on the side as gm/100ml
- Repeat the 5 to 6 times and take the average value

Packed Cell Volume (PCV) Determination (Ibra Bancroft and Gamble, 2002; Ibrahim, 2013)

- The blood is filled in to a micro hematocrit tube to ($3/4^{th}$) and seals it with sealer
- Centrifuge the filled hematocrit tube in a hematocrite centrifuge at 12000 rpm for 4 Minutes.
- Read the value with hematocrit reader and record the result

The Procedures of the Total RBC Count (Ibrahim, 2013)

- Take the blood in to RBC pipette up to 0.5 marks
- Immediately draw the RBC diluting fluid up to mark 101
- Rotate the pipette between thumb and other fingers with finger. This gives a dilution of 1:200.
- Clean the counting chamber of hemocytometer and cover slip
- Place the cover slip in position over the counting chamber by gentle pressure

- Expel a drop of blood on to the counting chamber by holding the pipette at an angle of 45^0.
- Allow the hemocytometer for 2-3 min to settle down the RBC in counting chamber

Counting rules

- Count under 40x objective under microscope
- Don't counting the cell touching the bottom and right lines
- Count first from left to right directions and then vice versa
- Counting the cell touching the left and tope lines

Calculations

- Total Erythrocyte count /TEC/cu.mm = Cells counted x 10 (0.1 mm depth) x 5(1/5 of sq.mm.)x200 (1:200 dilution)
- = Sum of cells in 5 small squaresx 10,000

The Procedures of Total White Blood Cell Count (Ibrahim, 2013)

- Draw the blood in to WBC pipette up to 0.5 marks
- Immediately draw the WBC diluting fluid up to 11 marks
- Rotate the pipette between thumbs and finger horizontally. This will gives you a dilution of 1:20.
- Clean the counting chamber of hematometer and cover slip.
- Place the cover slip on the counting chamber with gentle pressure
- Expel the fluid in the pipette by an angle of 45^0
- Allow the hemocytometer for 2 minute to settle down the WBC
- Count the WBC in the four large squares in the corners of counting chamber (16 small squares).

Counting

- Count cells touching the left and top side lines
- Don.t count cells touching the right and bottom side lines
- Avoid duplication of cells

Calculation

- Total WBC count = cells counted x 50

Annex II: Blood eosinophil determination Procedures

- Draw the blood from vacutainair tube into the pipette until slightly pass 0.5 graduation
- The pipette is then quickly withdrawn and the tip is wiped free of excess blood
- After drawing the blood, immerse the end of the pipette into the eosinophil diluting solution
- Then the pipette is held between the thumb and forefinger and shake slowly for approximately 10 seconds
- The chambers was loaded by hold the pipette at a 45 degree angle, discharge three drops of the solution to empty the capillary stem
- Then place the tip of the pipette on the chamber to be loaded
- Allow the fluid to run out rapidly and fill the chamber without overflowing at the edges
- Then allow the loaded counting chamber to stand for at least three minutes to permit lysis, staining and settling of the cells
- Count the eosinophil in 4x4 grid counting chamber with 40x magnification power of light microscope

Calculation

- Counts/ml $= \frac{\text{Total counts(N)x Dilution factor(eg. 100) x}10^4}{\text{Number of 4x4 grids counted}}$

Annex III: Histopathological Procedures (Takulder, 2007)

- **Fixation** of tissue by 10% neutral buffered formaldehyde
- Trimming part of the tissue in a way that the lesion we require be included or not missed and to fit standard histological processing tissue cassettes (5mm thickness).
- Tissue specimen processing: fixation of tissue by formalin, dehydrating tissue by increasing alcohols concentration, clearing of tissue by xylene, and impregnation of tissue by paraffin wax. Formalin-I 2hr - ---Formalin-II 2hr - --70% Alcohol 1hr --- 95% Alcohol- 100% Alcohol-I1hr--- 100% Alcohol-II 2hrs ----- 100%

Alcohol-III 2hrs ----- Xylene-I 1:30hrs ------- Xylene-II 1:30hrs ------ Xylene-III 1:30hrs ------ Paraffin-I 2hrs --- Paraffin-II 3hrs.

- **Embedding of processed tissue**: impregnated tissue is placed in a mould with their labels and then fresh melted wax (54-60^{0}c) is poured and allowed to settle and solidify.
- **Sectioning**: sectioning of tissue in 3-5 micron thickness and put on water bath to straighten the ribbon, and then adhere on the surface of frost ended and clear slide. Later label and put an incubator overnight.
- **Staining**: Hematoxyline eosine staining procedure
    - Deparaffinize slides in two changes of xylene for 5minutes
    - Hydrate slides in three changes of 100% alcohol each for 3minutes and 1 changes of 95% alcohol for a minute and one change of 70% alcohol for 3minutes
    - Rinse in distilled water until repples disappear from slides.
    - Place in heamatoxyline (mayer's hematoxline) for 10-15 minutes
    - Rinse in tape water until water runs clear
    - Decolorize in 1% acid alcohol (Eosin), 3 qiuck dips. Check differentiation microscopically: Nucleic should be distinct; cytoplasm should be uncolored.
    - Rinse in tap water until ripples disappear from slides. Stain in eosin, 3 dips.
    - Dehydrate in 70% 3 dips, 95% alcohol of 3dips and 100% alcohol, 3 changes each 3minutes.
    - Clear in 3 changes of xylene for 5 minutes each. Mount cover glass with DPX.
    - Examination of the prepared slides under the microscope

Annex IV: Coprological Methods

Procedure of Floatation method:

- Place about 2gram of the fecal sample in a suitable container such as cup.

- Add 30ml of floatation fluid and make an emulsion by mixing the fluid with the feces
- It was strained through a metal tea strainer into second cup
- The floatation fluid is added in to the test tube until a meniscus is formed
- Placed a glass coverslip over the meniscus and allowed to remain for 10-15 minutes
- Then coverslip is removed and placed on slide and examine under the microscope.

Procedure of Sedimentation Techniques:

- Mix two gram of fecal with tap water in a cup
- The mixture is strained by a tea strainer into a centrifuge tube.
- The sample is centrifuged at about 1500 cycle/ minute (rpm)
- Pour off the solution in the top of the tube without disturbed the sediment at the bottom.
- Transfer a small amount of the top layer of the sediment to a slide with pipette and bulb
- The cover slip is placed to the drop and examine under the microscope

Procedures of Baermann Techniques:

- Prepared and spread a piece of gauze and place 5gram of fecal on the gauze
- Then after covered by the warm water
- Allow the feces on the apparatus to remain undistributed overnight
- Placed the petridish under the rubber tube, and open the plug to allow a large drop of fluid to fall on the slide
- A coverslip is applied to the slide and examined microscopically for the presence of larvae

Annex V: Recording Sheets of lesions, recovered worms, hematological and serum biochemical data during research

Recording Sheets of lesions and recovered adult worms

No	Spp code	Spp of animals	Worm count	Site of infection	Gross lesion	Tissue sample

Hematological parameters of infected and uninfected control sheep and goat

Animal code	PCV (%)	Hb(g/dl)	RBC x10^6	WBC x10^3	Blood indices			Differential WBC Count				
					MCV (fl)	MCH (pg)	MCHC (g/dl)	N %	E %	B %	M %	L %

Annex VI: Different pictures captured during the study period

Figure: Captured during hematological investigation in the laboratory

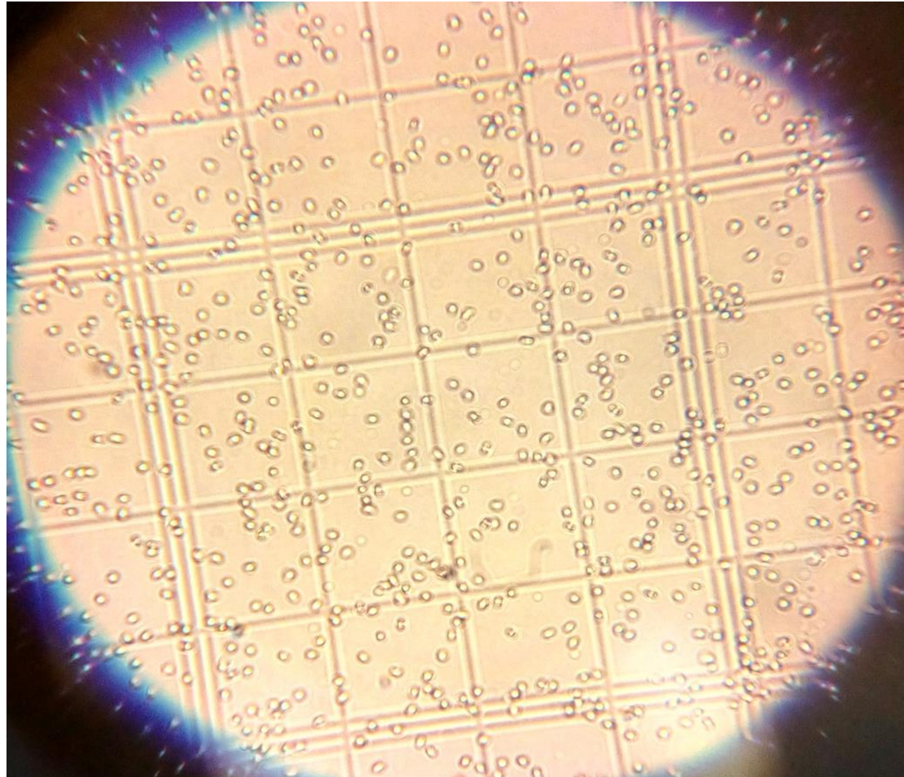

Figure: RBCs picture captured from light microscope in 40x magnification power from hem-cytometer chamber

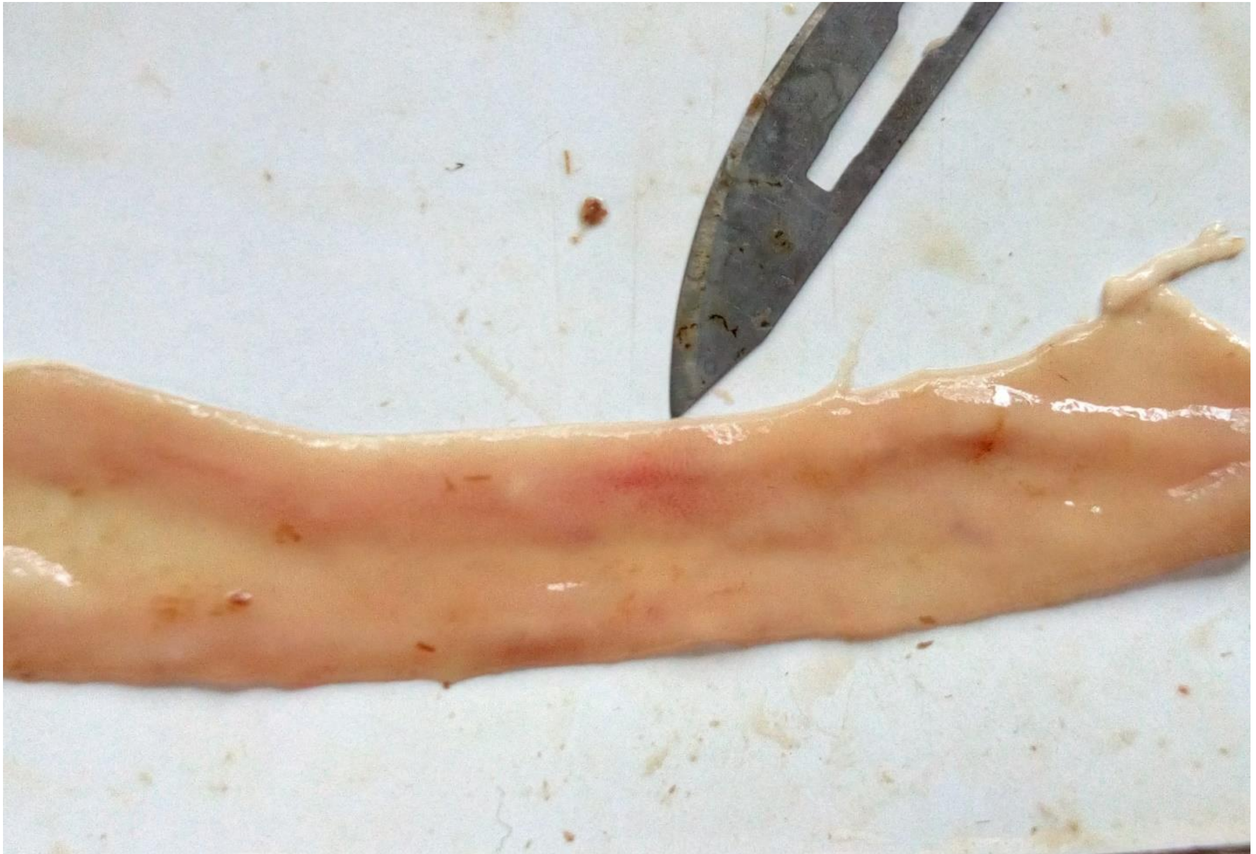

Figure: small intestine macroscopic lesion with petechial hemorrhage (indicated by blade)

Figure: Small intestine captured on the tray during post mortem examination

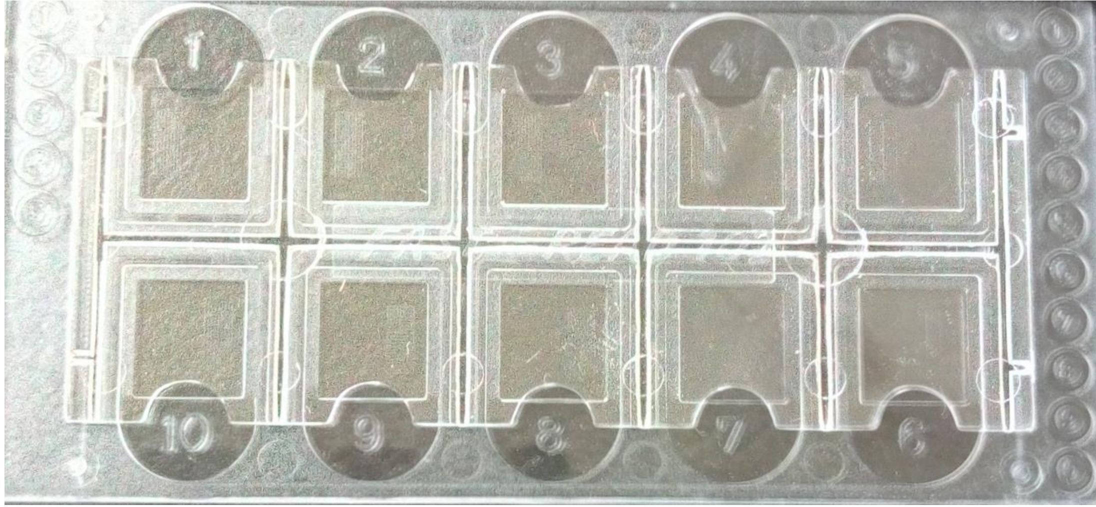

Figure: Fast Read 102 Counting Chambers of blood eosinophil per ml